MOLECULAR PHYLOGENETIC INVESTIGATION OF THE CLINGFISHES

(TELEOSTEI: GOBIESOCIDAE)

A Thesis

by

CRAGEN DANIELLE KING

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Chair of Committee,	Kevin W. Conway
Committee Members,	Gary Voelker
	David Portnoy
Interdisciplinary Faculty Chair,	Duncan MacKenzie

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ABSTRACT

Currently, there are roughly 170 species of clingfishes (family Gobiesocidae) divided between ten subfamilies in a "phenetic" classification scheme proposed over 60 years ago. Recently, an alternative classification scheme was proposed which included only two subfamilies. For this study, a large scale multi-locus investigation on the phylogenetic relationships of the Gobiesocidae was conducted using both mitochondrial and nuclear DNA sequence data to assess whether the two available classification schemes reflect the evolutionary relationships of the group. Phylogenetic hypotheses are obtained from Bayesian and Maximum Likelihood analyses of two mitochondrial (12S and COI; 1062 bp) and five nuclear genes (ENC1, GLYT, MYH6, SH3PX3, and ZIC1; 3785 bp) for 81 species of clingfishes. Four of the ten subfamilies (Aspasminae, Diademichthyinae, Diplocrepinae, and Gobiesocinae) and four genera (*Aspasmichthys, Cochleoceps, Lepadichthys,* and *Lepadogaster*) are obtained as not monophyletic. The resulting topologies also do not recover the two-subfamily classification scheme as useful for classifying clingfishes because subfamily Cheilobranchinae is obtained as a monophyletic group that is deeply embedded inside the second subfamily Gobiesocinae. The two available classification schemes and their included subfamilies are discussed in detail.

DEDICATION

I dedicate this thesis to my grandparents, parents, siblings, and to the Smiths for all of their love and support. And to the clingfishes. Keep holding on.

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1. INTRODUCTION

Clingfishes of the family Gobiesocidae are distributed worldwide in tropical and temperate regions of the Atlantic, Pacific, and Indian oceans (Briggs, 1955). Clingfishes are common inhabitants of the intertidal zone where they reside among boulder fields, seagrass meadows, or coralline rubble, and a few are even commensal with echinoderms (Briggs, 1955; Patzner, 1999; Hofrichter & Patzner, 2000; Craig & Randall 2008; Fricke, 2014). Though these small, benthic fishes are found predominantly in shallow marine coastal waters, six are known from deeper coastal regions (100-350m depths) in the Caribbean, Australasia, and Japan (Fricke et al.; 2016; Hastings & Conway, 2017), and seven dwell in swift-flowing freshwater coastal streams in Central and South America (Briggs, 1955; Conway et al., 2017a). Gobiesocids possess an intriguing well-developed ventral adhesive disc composed of elements of the paired fin girdles (Guitel, 1888). Papillae located on the ventral surface of the disc, as well as their flattened form, allow clingfishes to adhere to even irregular and heavily fouled substrates and maintain this position against the ebb and flow of the current with adhesive forces 80-230 times the body weight of the fish (Ditsche et al., 2014; Wainwright et al., 2013).

The approximately 170 species of clingfishes placed within the Gobiesocidae were originally divided between eight subfamilies (Aspaminae, Chorischismінae, Diademichthyinae, Diplocrepinae, Haplocyclicinae, Gobiesocinae, Lepadogastrinae, and Trachelochismінae) based on a three-character system introduced by Briggs (1955). This system groups the different species of clingfishes together into subfamilies based on the

type of adhesive disc present (single or double), the number of gill arches baring filaments (3 or 4), and the arrangement of the gill membranes (united with or free from the isthmus). Subsequent to Briggs (1955), Springer and Fraser (1976) transferred the Australian shore eels of the genus *Alabes* from the family (Cheilobranchidae) to the Gobiesocidae, which resulted in a ninth subfamily (Cheilobranchinae) being added to the classification for the family. Recently, Fricke et al. (2017) erected a tenth subfamily, Protogobiesocinae, for the supposedly "laterally asymmetrical" *Protogobiesox* (Fricke et al., 2017) and *Lepadicyathus* (Prokofiev, 2005).

In his monographic revision of the Gobiesocidae Briggs (1955) recognized 30 genera and 85 species. Since 1955, multiple new species and new genera of gobiesocids have been described and currently, 48 genera (Table 1) and 171 species are considered valid (Eschmeyer and Fong, 2017). With the continuing discovery of new species it has become increasingly difficult to place new taxa into the subfamilies established by Briggs (1955), leading to heavy criticism (Smith, 1957; Böhlke & Robins, 1970; Springer and Fraser, 1976; Hardy, 1983; Allen, 1984; Almada et al., 2008; Craig & Randall, 2009, Conway et al., 2015). Other morphological characteristics (beyond those used by Briggs) are often disregarded when new taxa are added to an existing subfamily and in many cases, these the new additions differ markedly from the remaining members. For example, *Modicus* was placed in Aspasminae but differs from all genera of the group in the number of gill rakers and pectoral-fin ray, and features of oral dentition (Hardy, 1983). Also, *Derilissus* was added to the Gobiesocinae by Briggs

(1969) even though it exhibits the characters of the Diademichthyinae (Böhlke & Robins, 1970).

Table 1. Subfamilies and included genera of the Gobiesocidae. Generic assignment to subfamilies is based on Briggs (1955). An asterisk (*) accompanies those subfamilies and genera erected subsequent to Briggs (1955) (e.g., *Gymnoscyphus* was placed in the Haplocylicinae by Böhlke & Robins, 1970). The genus *Gastrocymba* was included in the Trachelochisminae by Briggs (1955) but is listed here under the Diplocrepinae following Hardy (1984). The genus *Lepadicyathus* was included in the Aspasminae by Prokofiev (2005) but is listed here under the Protogobiesocinae following Fricke et al. (2017). The genus Pherallodiscus was synonymized with the genus *Gobiesox* of the Gobiesocinae by Conway et al. (2017).

Subfamily	Included Genera	References
Aspasminae	*Aspasma, Aspasmichthys, Aspasmodes*, Briggsia*, Liobranchia, Lissonanchus*, Modicus*, Pherallodichthys*, Posidonichthys**	Smith, 1957; Smith, 1965; Hardy, 1983; Shiogaki & Dotsu 1983; Briggs, 1993; Craig & Randall, 2009
Cheilobranchinae*	*Alabes**	Springer and Fraser, 1976; Briggs, 1993
Chorisochisminae	*Chorisochismus*	
Diademichthyinae	*Diademichthys, Discotrema*, Lepadichthys, Unguitrema**	Briggs, 1976; Fricke, 2014
Diplocrepinae	*Aspasmogaster, Cochleoceps, Diplocrepis, Gastrocyathus, Gastrocymba*, Gastroscyphus, Parvicrepis, Pherallodus, Propherallodus**	Shiogaki & Dotsu, 1983; Springer and Fraser, 1976
Gobiesocinae	*Acyrtops, Acyrtus, Arcos, Derilissus*, Eckloniaichthys, Gobiesox, Pherallodiscus (= Gobiesox)*, Rimicola, Sicyases, Tomicodon*	Briggs, 1969; Conway et al., 2017a
Haplocyclicinae	*Haplocylix, Gymnoscyphus**	Böhlke & Robins, 1970
Lepadogastrinae	*Apletodon, Diplecogaster, Gouania, Lepadogaster, Lecanogaster*, Opeatogenys*	Briggs, 1957
Protogobiesocinae*	*Lepadicyathus*, Protogobiesox**	Prokofiev, 2005; Fricke et al., 2017
Trachelochisminae	*Conidens, Creocele, Dellichthys, Kopua*, Trachelochismus*	Hardy, 1984

Studies on the phylogenetic relationships of clingfishes are scarce. Conway et al. (2017a), the most comprehensive study to date, included thirty-two taxa and multiple loci in a molecular phylogenetic investigation of the New World clingfishes (members of the Gobiesocinae) and representatives from five Old World subfamilies

(Cheilobranchinae, Chorisochisminae, Diademichthyinae, Diplocrepinae, and Lepadogastrinae). Earlier studies had fewer species (only thirteen in Conway et al., 2014; eleven in both Almada et al. [2008]; Fricke et al. [2017], and several of these studies (e.g., Conway et al., 2014; Fricke et al., 2017) did not recover any relationships that would challenge Briggs' (1955) classification scheme.

An alternative classification scheme comprised of two subfamilies was utilized by Van der Laan et al. (2014), which divides gobiesocids between the Cheilobranchinae (including *Alabes* only) and the Gobiesocinae (including all remaining genera). This alternative classification scheme may also be problematic because previous studies have obtained *Alabes* deeply embedded within the Gobiesocidae and not as the sister taxon to the remaining species (Conway et al., 2017a; Fricke et al., 2017). Though embraced by some (e.g., Nelson et al., 2016), the majority of researchers working with these captivating fishes continue to classify new species following the classification scheme of Briggs (e.g., Fricke et al. 2017) or refrain from making subfamily level designations entirely (e.g., Conway et al., 2017a). Clearly, a large, intensive phylogenetic study is needed for the Gobiesocidae to better understand intrarelationships and how these relationships correlate with the two currently available classifications.

Here, I conducted a large-scale, multi-locus investigation of the phylogenetic relationships of the Gobiesocidae. My main objective was to assess whether the two current alternative classification schemes accurately reflect the evolutionary relationships of the group.

2. MATERIALS AND METHODS

2.1 Taxon sampling

The ingroup is comprised of 81 species (5 undescribed), representing 42 genera (5 undescribed) and all 10 subfamilies of the Gobiesocidae (Briggs, 1955; Fricke et al., 2017; Table 2). Three members of the Blenniidae (*Ophioblennius atlanticus, Salarius fasciatus,* and *Entomacrodus nigricans*), one member of the Grammatidae (*Gramma loreto*), and one member of the Pseudochromidae (*Labracinus cyclophthalmus*) were included as outgroups based on their placement as putative close relatives of Gobiesocidae within the Ovalentaria or "egg-filament clade" (Wainwright et al., 2012; Near et al., 2013; Eytan et al., 2015) and the use of these species as outgroups in a previous molecular phylogenetic study on clingfishes (Conway et al., 2017a).

Table 2. Family and subfamily designations, museum voucher numbers, and Genbank accession numbers of taxa included in this study.

Family	Subfamily	Genus	Species	Museum Voucher	COI	12S	ZIC1	SH3PX3	GLYT	ENC1	MYH6
Gobiesocidae	Chorisochisminae	*Chorisochismus*	*dentex*	TCWC 16779.01	KY656439	KY656410	KY686191	KY686158	KY686093	xxxxx	KY686125
Gobiesocidae	Cheilobranchinae	*Alabes*	*dorsalis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Cheilobranchinae	*Alabes*	*hoesei*	AMS I.44632-019	KY656441	KY656412	KY686193	KY686160	KY686095	xxxxx	KY686127
Gobiesocidae	Haplocylinae	*Haplocylix*	*littoreus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Protogobiesocinae	*Protogobiesox*	*asymmetricus*	xxxxx	KY126071.1	KY126079.1	-	-	-	-	-
Gobiesocidae	Gobiesocinae	*Acyrtus*	*artius*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Gobiesocinae	*Acyrtus*	*lanthanum*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Acyrtus*	*rubiginosus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Acyrtops*	*beryllinus*	TCWC 15701.01	KJ616449.1	KY656399	KY686180	KY686147	KY686086	xxxxx	KY686115
Gobiesocidae	Gobiesocinae	*Arcos*	*erythrops*	SIO-01-182	KY656430	KY656400	KY686181	KY686148	KY686085	xxxxx	KY686114
Gobiesocidae	Gobiesocinae	*Derilissus*	sp.	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Gobiesox*	*adustus*	SIO 01-182	KY656417	KY656384	KY686165	KY686132	KY686071	xxxxx	KY686099
Gobiesocidae	Gobiesocinae	*Gobiesox*	*barbatulus*	TCWC uncat.	KY656418	KY656385	KY686166	KY686133	KY686072	xxxxx	KY686100
Gobiesocidae	Gobiesocinae	*Gobiesox*	*cephalus*	STRI_04273	KY656421	KY656388	KY686169	KY686136	KY686077	xxxxx	KY686106
Gobiesocidae	Gobiesocinae	*Gobiesox*	*daedelus*	SIO 03-42	KY656419	KY656386	KY686167	KY686134	KY686073	xxxxx	KY686101
Gobiesocidae	Gobiesocinae	*Gobiesox*	*funebris*	SIO 02-1	KY656429	KY656397	KY686178	KY686145	KY686083	xxxxx	KY686112
Gobiesocidae	Gobiesocinae	*Gobiesox*	*juradoensis*	SIO-03-42-1	KY656423	KY656391	KY686172	KY686139	KY686075	xxxxx	KY686103
Gobiesocidae	Gobiesocinae	*Gobiesox*	*maeandricus*	VIMS 12257	KJ616451.1	KY656390	KY686171	KY686138	KY686076	xxxxx	KY686104
Gobiesocidae	Gobiesocinae	*Gobiesox*	*mexicanus*	TCWC uncat.	KY656428	KY656396	KY686177	KY686144	KY686082	xxxxx	KY686111
Gobiesocidae	Gobiesocinae	*Gobiesox*	*nigripinnis*	TCWC uncat.	KY656422	KY656389	KY686170	KY686137	-	xxxxx	KY686105
Gobiesocidae	Gobiesocinae	*Gobiesox*	*pinniger*	SIO-06-51-1	KY656426	KY656394	KY686175	KY686142	KY686080	xxxxx	KY686109
Gobiesocidae	Gobiesocinae	*Gobiesox*	*potamius*	STRI_04281	KY656425	KY656393	KY686174	KY686141	KY686079	xxxxx	KY686108
Gobiesocidae	Gobiesocinae	*Gobiesox*	*punctulatus*	TCWC 16452.03	KY656424	KY656392	KY686173	KY686140	KY686078	xxxxx	KY686107
Gobiesocidae	Gobiesocinae	*Gobiesox*	*rhessodon*	SIO-09-170-2	KY656427	KY656395	KY686176	KY686143	KY686081	xxxxx	KY686110
Gobiesocidae	Gobiesocinae	*Gobiesox*	*strumosus*	TCWC uncat.	KY656420	KY656387	KY686168	KY686135	KY686074	xxxxx	KY686102
Gobiesocidae	Gobiesocinae	*Rimicola*	*muscarum*	SIO 03-40	KJ616452.1	KY656398	KY686179	KY686146	KY686084	xxxxx	KY686113
Gobiesocidae	Gobiesocinae	*Sicyases*	*sanguineus*	ANSP 191454	KJ616453.1	KY656401	KY686182	KY686149	KY686087	xxxxx	KY686116
Gobiesocidae	Gobiesocinae	*Tomicodon*	sp.	SLU1553	KY656435	KY656406	KY686187	KY686154	-	xxxxx	KY686121
Gobiesocidae	Gobiesocinae	*Tomicodon*	*boehlkei*	SIO-07-49	KY656431	KY656402	KY686183	KY686150	KY686088	xxxxx	KY686117
Gobiesocidae	Gobiesocinae	*Tomicodon*	*briggsi*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Tomicodon*	*humeralis*	SIO-02-1	KY656432	KY656403	KY686184	KY686151	-	xxxxx	KY686118
Gobiesocidae	Gobiesocinae	*Tomicodon*	*lavettsmithi*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Tomicodon*	*myersi*	SIO-07-2-1	KY656433	KY656404	KY686185	KY686152	-	-	KY686119
Gobiesocidae	Gobiesocinae	*Tomicodon*	*reitzae*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Gobiesocinae	*Tomicodon*	*zebra*	SIO-07-2-V	KY656434	KY656405	KY686186	KY686153	KY686089	xxxxx	KY686120
Gobiesocidae	Gobiesocinae	*Eckloniaichthys*	*scylliorhiniceps*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Apletodon*	*dentatus*	TCWC uncat.	KJ616456.1	KY656415	KY686196	KY686163	KY686097	xxxxx	KY686130

Table 2 Continued

Family	Subfamily	Genus	Species	Museum Voucher	COI	12S	ZIC1	SH3PX3	GLYT	ENC1	MYH6
Gobiesocidae	Lepadogastrinae	*Apletodon*	*incognitus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Diplecogaster*	*bimaculata*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Gouania*	*willdenowi*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Lepadogaster*	*candollei*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Lepadogaster*	*lepadogaster*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Lepadogastrinae	*Lepadogaster*	*purpurea*	TCWC uncat.	KJ616457.1	KY656416	KY686197	KY686164	KY686098	xxxxx	-
Gobiesocidae	Lepadogastrinae	*Opeatogenys*	*gracilis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diademichthyinae	*Diademichthys*	*lineatus*	TCWC uncat	KY656436	KY656407	KY686188	KY686155	KY686090	xxxxx	KY686122
Gobiesocidae	Diademichthyinae	*Discotrema*	*crinophila*	TCWC uncat	KY656438	KY656409	KY686190	KY686157	KY686092	xxxxx	KY686124
Gobiesocidae	Diademichthyinae	*Lepadichthys*	*akiko*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diademichthyinae	*Lepadichthys*	*coccinotaenia*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diademichthyinae	*Lepadichthys*	*frenatus*	JFBM 46744	KY656437	KY656408	KY686189	KY686156	KY686091	xxxxx	KY686123
Gobiesocidae	Diademichthyinae	*Lepadichthys*	*lineatus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Aspasminae	*Aspasma*	*minima*	xxxxx	xxxxx	xxxxx	-	-	-	-	-
Gobiesocidae	Aspasminae	*Aspasmichthys*	*alorensis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Aspasminae	*Aspasmichthys*	*ciconiae*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Aspasminae	*Posidonichthys*	*hutchinsi*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Conidens*	*laticephalus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Creocele*	*cardinalis*	xxxxx	-	xxxxx	xxxxx	-	xxxxx	-	xxxxx
Gobiesocidae	Trachelochisminae	*Dellichthys*	*morelandi*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Dellichthys*	sp.	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Tracheloschismus*	*aestuarium*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Tracheloschismus*	*melobesia*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Trachelochisminae	*Tracheloschismus*	*pinnulatus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Aspasmogaster*	*costata*	AMS I.44625-043	KY656442	KY656414	KY686195	KY686162	KY686096	xxxxx	KY686129
Gobiesocidae	Diplocrepinae	*Aspasmogaster*	*liorhynchus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Aspasmogaster*	*tasmaniensis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Cochleoceps*	*bassensis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Cochleoceps*	*orientalis*	AMS I.41084-007	KY656440	KY656411	KY686192	KY686159	KY686094	xxxxx	KY686126
Gobiesocidae	Diplocrepinae	*Cochleoceps*	*spatula*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Cochleoceps*	*viridis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Diplocrepis*	*puniceus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Gastrocymba*	*quadriradiata*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Gastrocyathus*	*gracilis*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx
Gobiesocidae	Diplocrepinae	*Gastroscyphus*	*hectoris*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Parvicrepis*	*parvipinnis*	AMS I.43799-001	KJ616458.1	xxxxx	KY686194	xxxxx	-	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Parvicrepis*	sp.	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	xxxxx	xxxxx
Gobiesocidae	Diplocrepinae	*Pherallodus*	*indicus*	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-	-	xxxxx

Table 2 Continued

Family	**Subfamily**	**Genus**	**Species**	**Museum Voucher**	**COI**	**12S**	**ZIC1**	**SH3PX3**	**GLYT**	**ENC1**	**MYH6**
Gobiesocidae	Diplocrepinae	*Propherallodus*	sp.	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	-	Genus A		xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	-	Genus B		xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	-
Gobiesocidae	-	Genus C		xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	-	New Genus	"Urchin Clingfish"	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Gobiesocidae	-	New Genus	"Kermadec Clingfish"	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
Blenniidae	-	*Ophioblennius*	*atlanticus*	-	JQ842259	DQ143877	JX189136	JX189503	JX188810	JX188983.1	JX189749
Blenniidae	-	*Salarius*	*fasciatus*	-	NC_004412	NC_004412	KC830868	-	KF139872	KF678487.1	KF678589
Blenniidae	-	*Entomacrodus*	*nigricans*	-	JQ840835	DQ143880	KF140535	KF141465	-	KF678528.1	KF139990
Grammatidae	-	*Gramma*	*loreto*	-	JQ842131	-	JX189051	JX189578	JX188719	JX188886.1	JX189668
Pseudochromidae	-	*Labracinus*	*cyclophthalmus*	-	FJ583585	NC_009054	JX189076	JX189608	JX188751	JX188917.1	JX189692

2.2 DNA extraction and PCR amplification

Total genomic DNA was extracted from fin clip or muscle tissue (see Table 1 for source of tissues) with a DNeasy Blood and Tissue Extraction Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. Partial sequences were amplified for 12S ribosomal RNA (12S) and cytochrome c oxidase subunit I (CO1) using primers obtained from Kocher et al. (1989), Folmer et al. (1994) and/or Ward et al. (2005). An additional five protein-coding nuclear loci (zic family member 1 [ZIC1]; SH3 and PX domain containing 3 gene [SH3PX3]; glycosyltransferase [GLYT]; ectodermal-neural cortex 1 [ENC1]; cardiac muscle myosin heavy chain 6 alpha [MYH6]) were amplified with primers obtained from Li et al. (2007). PCR reactions were conducted in a 25 μl reaction containing 12.5 μl of EmeraldAmp® GT PCR Master Mix (Takara Bio USA Inc., Mountain View, CA, USA), 0.4 μl of each primer (10 μM), 10.7 μl of ddH2O, and 1 μl of DNA template. The mitochondrial loci were amplified using the reaction conditions described in Conway et al. (2017a). The nested PCR method and reactions conditions described in Li et al. (2007) were utilized for amplification of the nuclear loci. Negative controls were included to confirm the absence of contaminants. PCR products were purified and sequenced using high-throughput sequencing facilities at Beckman Coulter Genomics (Danvers, MA, USA) or the Genetics Core Lab of Texas A&M University-Corpus Christi (Corpus Christi, TX, USA). Additional sequences were also obtained from GenBank (Table 2).

2.3 Sequence alignment and phylogenetic analyses

Sequences were assembled and translated into amino acids for protein-coding genes in Geneious v.10.2.3 (Kearse et al., 2012). Individual gene alignments were conducted in MACSE (Ranwez et al., 2011) for protein-coding genes and MAFFT v.7.0 (Katoh & Standley, 2013) for the non-coding gene 12S. Resulting alignments were checked for accuracy manually in Mesquite v.3.2.0 (Maddison & Maddison, 2011). Aligned datasets were concatenated into three separate datasets using SequenceMatrix v.1.8 (Vaidya et al., 2011), including: (1) mtDNA dataset (comprising 12S + CO1); (2) nDNA dataset (comprising ZIC1 + SH3PX3 + GLYT + ENC1 + MYH6); and (3) combined dataset (included all 7 genes). Sequences representing the mitochondrial loci only were obtained for *Protogobiesox asymmetricus* and *Aspasma minima* and these were excluded from the combined dataset. The combined dataset was 93% complete with coverage for individual genes varying between 84–100% (refer to Table 3). Base pair composition and alignment length are summarized for both individual aligned gene datasets and the three concatenated datasets in Table 3.

Table 3. Summary of individual and concatenated datasets, including the number of taxa, alignment length, base pair composition, and best fitting substitution model.

	Dataset									
	Combined	**mtDNA**	**nDNA**	**COI**	**12S**	**ZIC1**	**SH3PX3**	**GLYT**	**ENC1**	**MYH6**
Number of Taxa	84	86	84	85	86	84	82	75	72	82
Alignment	4847	1062	3785	684	378	831	678	852	756	668
Average Empirical base pair composition (%)										
A	25.3	25.5	25.3	23.1	29.8	23.8	25.8	25.2	24.8	27.2
T	21.2	28.0	19.1	31.6	21.3	17.4	19.5	19.4	21.4	18.2
G	25.6	19.7	27.4	18.0	23.0	27.5	26.3	28.7	26.9	27.2
C	27.9	26.8	28.3	27.3	25.9	31.3	28.4	26.7	26.9	27.4
Constant sites	2741	501	2238	352	149	615	434	326	495	368
Parsimony uninformative sites	2079	555	1526	332	223	216	244	508	261	297
Parsimony informative sites	1696	477	1221	296	181	152	194	417	213	245
Best substitution model										
IQ TREE				TIM2+R6	TIM2+R5	TN+R3	TIM+I+G4	HKY+R3	TN+I+G4	TIM3e+R4
jModelTest				010023+I+G	TIM2+I+G	TrN+I+G	TIM1+I+G	HKY+I+G	TrN+I+G	TIM3ef+I+G

The best-fitting model of nucleotide substitution for the Bayesian analyses for each gene was assessed via the Bayesian Information Criteria (BIC) using jModeltest 2.1.7 (Darriba et al., 2012; Guindon and Gascuel, 2003) and ModelFinder (Kalyaanamoorthy et al., 2017; Chernomor et al., 2016) in IQTREE v.1.5.5 (Nguyen et al., 2015; Trifinopoulos et al., 2016) for the Maximum Likelihood (ML) analyses. Each dataset was partitioned by the models recovered for each molecular marker (Table 3).

For each individual gene and concatenated dataset, Bayesian analyses using MrBayes v.3.2.6 (Ronquist et al., 2012) were implemented in the CIPRES portal v.3.3 (Miller et al., 2010) and consisted of four independent runs of 50 million generations with four chains sampling every 1,000 generations. Tracer v.1.6 (Rambaut et al., 2014) was utilized to determine burn-in, check convergence and stationarity, and ensure adequate mixing of each chain (ESS > 200). Convergence was assessed by the potential scale reduction factor (PSRF) values in MrBayes and achieved when a PSRF value of 1.0 was obtained. After discarding a burn-in of 10%, the sampled trees were used to construct a 50% majority-rule consensus tree. ML analyses were executed in IQTREE (Nguyen et al., 2015) using the online portal with the data partitioned according to the models set by ModelFinder (Kalyaanamoorthy et al., 2017; Chernomor et al., 2016). IQTREE's ultrafast bootstrap (UFBoot) algorithm (Minh et al., 2013) was utilized to assess branch support using 1000 replicates. All resulting trees were rooted using *Labracinus cyclophthalmus.*

2.4 Species tree estimation

A species tree was constructed using StarBEAST (Bouckaert et al., 2014) in the CIPRES portal v.3.3 (Miller et al., 2010) using the best-fitting substitution models obtained for each gene (Table 3). Substitution and clock models were unlinked across all partitions and the population size model was set to linear. Four MCMC chains were run for 700 million generations, sampling every 10,000 generations. Convergence, stationarity, and burn-in were assessed using Tracer v.1.6 (Rambaut et al., 2014). TreeAnnotator v.2.4.6 (Drummond & Rambaut, 2007) was used to retrieve the maximum clade credibility tree from the accumulation of sampled species trees. All final trees were rooted using *Labracinus cyclophthalmus* and edited in Figtree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

2.5 Tests of alternative hypotheses

Two constrained topologies, compatible with each of the alternative classification schemes currently in use for the Gobiesocidae (Briggs, 1955; Nelson et al., 2016), were assembled in Mesquite v.3.2.0 (Maddison & Maddison, 2011) using the combined dataset excluding the five undescribed taxa. The two constrained topologies were tested against the topology resulting from a ML analysis (parameters as described above) of an altered version of the combined dataset (excluding the five undescribed taxa) using three likelihood ratio tests, including: (1) Kishino-Hasegawa (KH) test (Kishino & Hasegawa, 1989); (2) Shimodaira-Hasegawa (SH) test (Ota et al., 2000); and the (3) Approximately Unbiased (AU) test (Shimodaira, 2002). All likelihood tests were based on ten thousand

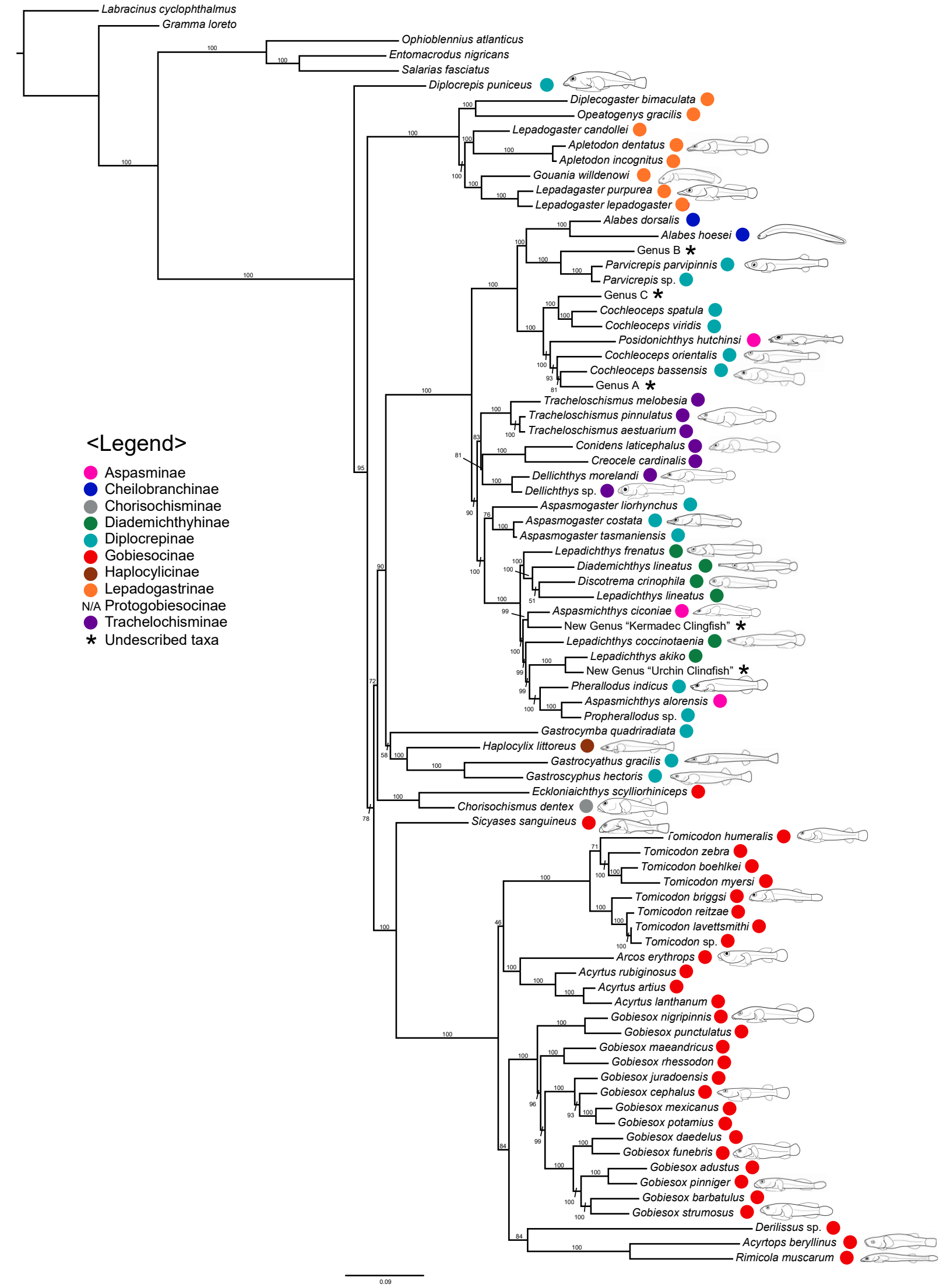
resamplings of the estimated log likelihoods (RELL method) as implemented in IQTREE.

3. RESULTS

3.1 Phylogenetic relationships

The relationships between taxa in the phylogenetic hypotheses resulting from the different analyses (Bayesian or ML) of the different datasets were largely incongruent. However, the relationships between taxa were mostly consistent between the gene trees resulting from the analyses of the nDNA datasets (Appendix C) and between the gene trees resulting from the analyses of the mtDNA datasets (Appendix B). There was also discordance between the topologies resulting from the phylogenetic analyses of the individual gene datasets surrounding the deeper phylogenetic relationships within the ingroup and on which species represent the sister group to the remaining gobiesocids. The species tree obtained for the combined dataset (Appendix F) was largely congruent with the topologies obtained from the ML and Bayesian analyses of the same dataset (Fig. 1 & Appendix A). In the following sections, I focus primarily on the topologies obtained from the analyses of the combined dataset (nDNA + mtDNA), which were largely congruent with the topologies resulting from the analyses of the nDNA dataset, and make reference to results obtained from the separate analyses of the nDNA, mtDNA, and individual gene datasets where conflict is present.

Figure 1. Phylogram obtained from the Maximum Likelihood analysis of the combined dataset labeled according to the subfamilies in the classification scheme established by Briggs (1955) with the addition of the Cheilobranchinae. Numbers above branches represent bootstrap support values.



The monophyly of the Gobiesocidae received strong statistical support in analyses of the combined (Figs. 1 & Appendix A) and nDNA datasets (Appendix C) (posterior probability [P.P.] = 1.0; bootstrap support [BS] = 100), but was not supported in any analyses of the mtDNA (Appendix B) or ENC1 datasets (Appendix D3 and E3). Strong statistical support was also obtained for the placement of *Diplocrepis puniceus* as the sister taxon to all remaining gobiesocids in the analyses of the nDNA dataset and the combined dataset (P.P. = 1.0; BS = 100). This relationship, however, was not present in the topologies resulting from the analyses of the individual gene datasets (Appendix D-E) or the mtDNA dataset (Appendix B). In the topologies resulting from the analyses of the combined dataset (Fig. 1 & Appendix A) the subfamilies Cheilobranchinae, Lepadogastrinae, and Trachelochisminae are obtained as monophyletic groups. A monophyletic Cheilobranchinae (represented by two species of *Alabes*) received strong statistical support in the Bayesian and ML analyses of the nDNA, mtDNA, and combined datasets (PP > 0.95; BS > 95) and the majority of the individual gene datasets, with lower statistical support in the analyses of ENC1 (PP = 0.62; BS = 89) and SH3PX3 (PP = 0.87; BS = 84). A monophyletic Lepadogastrinae (including members of *Apletodon, Lepadogaster, Gouania, Opeatogenys,* and *Diplecogaster*) received strong statistical support in the analyses of the combined and nDNA datasets (PP = 1.0; BS = 100) but was not recovered in any analysis of the mtDNA datasets (Appendix B). A monophyletic Trachelochisminae (including members of *Tracheloschismus, Dellichthys, Creocele,* and *Conidens*) received low statistical support in both the ML analysis of the combined dataset (BS = 83) and all analyses of the nDNA dataset (PP = 0.81; BS = 68).

The subfamilies Gobiesocinae, Diplocrepinae, Diademichthyinae, and Aspasminae were not recovered as monophyletic regardless of dataset or method of analysis. The remaining three subfamilies are either monotypic (Chorisochisminae) or the taxon sampling was not adequate enough to assess monophyly (Protogobiesocinae and Haplocylicinae).

All New World gobiesocids included for analyses (including members of *Acyrtus, Acyrtops, Arcos, Derilissus, Gobiesox, Rimicola, Sicyases,* and *Tomicodon*) were obtained as a monophyletic group with strong statistical support in topologies resulting from the analyses of the combined (Figs. 1 & Appendix A) and nDNA datasets (Appendix C) (PP = 1.0; BS = 100). The placement of *Sicyases sanguineus* was inconsistent between the topologies resulting from the analyses of different datasets. In addition to the combined and nDNA datasets, this species is placed as the sister taxon to the remaining members of the New World clade in topologies resulting from the analyses of the SH3PX3 and GLYT datasets (Appendix D4, D6, E4, E6) but is placed as the sister taxon to a large clade comprising members of the Lepadogastrinae, Gobiesocinae, Diplocrepinae, and Protogobiesocinae in the topologies resulting from the ML analyses of the 12S dataset (Appendix D1 & E1) (BS = 71) or as part of a larger polytomy comprised of multiple taxa in the topologies resulting from the Bayesian analyses of the 12S, ENC1, MYH6, and ZIC1 datasets (Appendix D1, D3, D5, D7). All members of the Aspasminae, Diademichthyinae, Diplocrepinae (excluding *Diplocrepis puniceus, Gastrocyathus gracilis, Gastroscyphus hectoris,* and *Gastrocymba quadriradiata*), and Trachelochisminae included in this study were obtained as a

monophyletic group with strong statistical support (P.P. > 0.90; BS > 95) in the topologies resulting from the analyses of the nDNA (Appendix C), mtDNA (Appendix B), and combined (Fig. 1 & Appendix A) datasets. This group was not obtained in the topologies resulting from the analysis of the individual CO1 and 12S datasets (Appendix D1-D2 and E1-E2) or the ML analysis of the ZIC1 dataset (Appendix B7). In the topology resulting from the ML analysis of the combined dataset (Fig.1), the two South African endemic taxa *Chorisochismus dentex* and *Eckloniaichthys scylliorhiniceps* are placed in a sister group relationship and together these two taxa represent the sister taxon to a clade comprised exclusively of the New Zealand endemic and obligate macrophytic algae inhabiting *Haplocylix littoreus, Gastrocyathus gracilis, Gastroscyphus hectoris, Gastrocymba quadriradiata* (BS = 72). This grouping is placed as the sister taxon to the aforementioned large monophyletic group comprising members of the Aspasminae, Diademichthyinae, Diplocrepinae, and Trachelochisminae (BS = 90). The topology resulting from the Bayesian analysis of the combined dataset (Appendix A) conflicted with that resulting from the ML analysis in the placement of the South African endemic clade (*C. dentex* + *E. scylliorhiniceps*) as part of a polytomy together also with the aforementioned New Zealand endemic macrophytic algae dwelling clingfishes, members of the Lepadogastrinae, the New World clade, and the larger monophyletic group comprising members of the Aspasminae, Diademichthyinae, Diplocrepinae, and Trachelochisminae (P.P. = 0.55).

Topologies resulting from the different analyses of the nDNA dataset (Appendix C) diverged from those obtained from the analyses of the combined dataset (Fig. 1 and

Appendix A). The ML analysis of the nDNA dataset (Appendix C2) obtained a topology that placed *Gastrocymba quadriradiata* as the sister taxon to a larger monophyletic group comprising members of the Aspasminae, Diademichthyinae, Diplocrepinae, and Trachelochisminae (BS = 48). The topology resulting from the Bayesian analysis of the nDNA dataset (Appendix C1) placed the South African endemic clade (*C. dentex* + *E. scylliorhiniceps*) as the sister taxon to a clade comprising members of the Lepadogastrinae (P.P. = 68), and these two together represent the sister taxon to a clade comprising members of the Aspasminae, Diademichthyinae, Diplocrepinae, and Trachelochisminae and the New Zealand endemic macrophytic algae dwelling clingfishes (P.P. = 0.98).

A number of the genera included for investigation were represented by multiple species, including: *Acyrtus* (3), *Alabes* (2), *Aspasmichthys* (2), *Aspasmogaster* (3), *Cochleoceps* (4), *Dellichthys* (2), *Gobiesox* (14), *Lepadichthys* (4), *Lepadogaster* (3), *Parvicrepis* (2), *Tomicodon* (8), and *Trachelochismus* (3). Members of *Acyrtus, Alabes, Aspasmogaster, Dellichthys, Gobiesox, Parvicrepis, Tomicodon,* and *Trachelochismus* were obtained as monophyletic groups in the majority of the resulting topologies from the analyses of the different datasets (excluding 12S, COI, ENC1, MYH6 [Bayes], and ZIC1 [ML]). *Aspasmichthys, Cochleoceps, Lepadichthys,* and *Lepadogaster* were never obtained as monophyletic groups regardless of the dataset or method of analysis.

3.2 Tests of alternative hypotheses

The alternative topologies tested were significantly different from the topology resulting from the analysis of the combined dataset (excluding the 5 undescribed taxa) (Table 4).

Table 4. Comparison of the likelihood values of the two constrained topologies against the ML combined topology excluding the five undescribed taxa. Plus signs denote the 95% confidence sets. Minus signs denote significant exclusion.

Tree	logL	deltaL	p-KH	p-SH	p-AU
Recovered topology	-179967.78	0.000	1.000 +	1.000 +	1.000 +
Briggs (1955)	-187426.31	7458.53	0.000 -	0.000 -	0.000 -
Nelson et al. (2016)	-187650.76	7682.98	0.000 -	0.000 -	0.000 -

4. DISCUSSION

4.1. Monophyly of Gobiesocidae and its subfamilies

The monophyly of the Gobiesocidae is well supported by morphological characters (Briggs, 1955; Wiley & Johnson, 2010) and the majority of previous molecular phylogenetic studies that have included two or more gobiesocid taxa for phylogenetic analysis have corroborated the monophyly of the group (e.g, Chen et al., 2003; Li et al., 2009; Conway et al., 2014; Eytan et al., 2015; Fricke et al., 2017; Conway et al., 2017a). In their investigation of the relationships of the eastern Atlantic lepadogastrine gobiesocids based on 12S and 16S mitochondrial ribosomal DNA, Almada et al. (2008) did not obtain a monophyletic Gobiesocidae due to the placement of the single outgroup taxon (*Parablennius pilicornis*; Blenniidae) within the ingroup clade. A similar situation in which non-gobiesocid outgroup taxa grouped with members of the ingroup was reported by Conway et al. (2017a) in the topologies resulting from the different analyses of the two mitochondrial loci used in that study (COI and 12S) and also herein in the topologies resulting from the analyses of the same loci (Appendix D1-D2 & E1-E2). Non-monophyly of the Gobiesocidae in each of these cases is likely an artifact associated with the analysis of smaller mtDNA datasets and is unlikely given the strong morphological evidence in support of gobiesocid monophyly and the results of other molecular studies (including the present study) that have utilized nuclear loci.

Depending on the classification scheme utilized, gobiesocids are divided either between two subfamilies (Gobiesocinae and Cheilobranchinae; Nelson et al., 2016) or

ten (Aspasminae, Cheilobranchinae, Chorisochisminae, Diademichthyinae, Diplocrepinae, Gobiesocinae, Haplocylicinae, Lepadogastrinae, Protogobiesocinae, and Trachelochisminae; Briggs, 1955; Springer & Fraser, 1976; Conway et al., 2015; Fricke et al., 2017) (Table 1). Though several previous investigations of clingfishes have raised concern over the composition of multiple subfamilies (e.g., Conway et al., 2017a), the phylogenetic studies that have been conducted to date have not been designed to adequately test the monophyly of individual subfamilies and as such have been limited in their ability to assess the utility of the alternative classification schemes. The majority of these studies have also employed non-overlapping taxon sampling schemes and have produced conflicting results. For example, in their phylogenetic investigation of the eastern Atlantic lepadogastrine clingfishes, Almada et al., (2008) included representatives of three subfamilies (Aspasminae, Gobiesocinae, and Lepadogastrinae) and obtained two (Gobiesocinae and Lepadogastrinae) as non-monophyletic. Fricke et al. (2017) included representatives of six subfamilies, including three represented by multiple individuals (Cheilobranchinae, Gobiesocinae, and Lepadogastrinae), all of which were obtained as monophyletic. In their molecular phylogenetic investigation of the New World clingfishes, Conway et al. (2017a) noted problems with each of the alternative classification schemes, including the non-monophyly of the Gobiesocinae (sensu Nelson et al., 2016) and the non-monophyly of at least the Diplocrepinae (sensu Briggs, 1955), but lacked representatives of several polytypic subfamilies (Aspasminae, Haplocylicinae, Protogobiesocinae, and Trachelochisminae) and were unable to comment on the monophyly or relationships of these taxa.

The taxon sampling of the present study greatly exceeds that of previous studies (81 species vs. 32 included in Conway et al., 2017a) and provides an opportunity not only to comment on the composition of several polytypic subfamilies that have been represented in previous studies only by a single taxon (e.g., Aspasminae) or missing entirely (e.g., Trachelochisminae) but also to more critically assess the monophyly of the remaining subfamilies based on an expanded data set. In the following sections, I discuss the generic composition of each of the ten subfamilies recognized currently in relation to the topologies obtained herein.

4.1a Subfamily Aspasminae

Briggs (1955) originally placed three genera into the Aspasminae, including *Aspasma, Aspasmichthys,* and *Liobranchia.* Several additional genera added subsequent to Briggs (1955), including *Aspasmodes* (Smith, 1957), *Briggsia* (Craig & Randall, 2009), *Lissonanchus* (Smith, 1965), *Modicus* (Hardy, 1983), *Pherallodichthys* (Shiogaki & Dotsu, 1983), and *Posidonichthys* (Briggs, 1993). Only four species, representing three genera of aspasmine clingfishes, were available for investigation herein. This includes *Aspasma minima* (mtDNA dataset only), *Aspasmichthys ciconiae*, *Aspasmichthys alorensis*, and *Posidonichthys hutchinsi*. Regardless of the dataset or method of analysis, these aspasmine taxa were never obtained as a monophyletic group. Instead, these taxa were consistently obtained as more closely related to members of other subfamilies than to each other. The southern Australian endemic *Posidonichthys hutchinsi* (Briggs, 1993; Hutchins, 2008) was obtained as a member of a monophyletic group comprised

exclusively of other southern Australia clingfishes, including the diplocrepines *Cochleoceps* and *Parvicrepis*, the cheilobranchine *Alabes*, and several undescribed species from this region currently referred to the undescribed genera A, B, and C (Hutchins, 2008) that are not currently assigned to any subfamily. Though Briggs (1955) placed *Posidonichthys* within the Aspasminae, he alluded to a possible close relationship with unnamed members of the Diplocrepinae based on shared characteristics of the adhesive disc that were not discussed. A close relationship between *Posidonichthys* and two other genera of southern Australian clingfishes (Genus A and *Nettorhamphos*) was recently hypothesized by Conway et al. (2017b) based on putatively derived characteristics of the subopercle. Though *Nettorhamphos* was not included in this study (due to a lack of appropriate tissue samples), *Posidonichthys* was obtained as the sister taxon to a clade comprising Genus A, *Cochleoceps orientalis*, and *Cochleoceps bassensis* (P.P. = 1; BS = 100).

The two species of *Aspasmichthys*, which were never obtained as sister taxa, were obtained as part of a larger monophyletic group comprising a number of Indo-Pacific clingfishes, including members of *Lepadichthys, Discotrema, Diademichthys, Pherallodus,* and *Propherallodus,* and the undescribed "Urchin Clingfish" and "Kermadec Clingfish". Within this larger group, *Aspasmichthys alorensis*, known from Japan, Taiwan, and South Korea (Briggs, 1955; Allen & Erdmann, 2012) was obtained in a well-supported sister group relationship with the undescribed "Kermadec Clingfish", a species known currently only from the remote Kermadec archipelago to the northeast of New Zealand (Stewart, 2015). *Aspasmichthys alorensis*, known only from a handful

of specimens collected recently from the Alor Strait of Indonesia (Allen & Erdmann, 2012) was obtained in a well-supported sister group relationship with *Propherallodus* sp. Allen & Erdmann (2012) "tentatively" assigned *Aspasmichthys alorensis* to *Aspasmichthys* based on characters listed in Briggs (1955) and described their decision as "provisional". Based on the results obtained herein, generic reassignment of *Aspasmichthys alorensis* may be warranted.

Only mtDNA sequences of *Aspasma minima* were available for investigation and in the topologies resulting from the analysis of the mtDNA dataset this species was obtained as the sister taxon to *Lepadichthys frenatus* (a member of the Diademichthyinae), within a clade comprising other Indo-Pacific clingfishes of the Aspasminae, Diplocrepinae, and Diademichthyinae, and the southern Australian endemic *Creocele cardinalis*. *Aspasma minima* has been included in two previous molecular phylogenetic investigations of clingfishes, including the study of Almada et al. (2008) and Fricke et al. (2017). Almada et al. (2008), obtained *Aspasma minima* in a poorly supported relationship with the eastern Atlantic lepadogastrine *Lepadogaster candollei* based on analyses of 12S and 16S mtDNA sequence data. Based on a combination of nDNA and mtDNA sequence data, Fricke et al. (2017) obtained *Aspasma minima* as the sister taxon to the Indo-Pacific diademichthyine *Lepadichthys lineatus.*

4.1b Subfamily Cheilobranchinae

The family Cheilobranchidae was synonymized with Gobiesocidae by Springer and Fraser (1976), resulting in the addition of the elongate Australian shore eels of the genus *Alabes* to the Gobiesocidae. Only two of the eleven currently recognized species of *Alabes* (*A. dorsalis* and *A. hoesei*) were available for investigation and, as in previous studies (Fricke et al., 2017), both were obtained as a monophyletic group regardless of the dataset or methods of analysis.

Springer and Fraser (1976) speculated that *Alabes* may be a close relative of *Gastrocymba quadriradiata,* a relatively elongate species of clingfish endemic to the sub-Antarctic region of New Zealand (Stewart, 2015). This putative relationship was based on a high number of osteological reductions shared by both taxa. A close relationship between *Alabes* and *G. quadriradiata* was not present in any of the topologies resulting from the different analyses of the different datasets. In all cases, *Alabes* was obtained as part of a monophyletic group comprised exclusively of other southern Australian taxa, including species of *Cochleoceps* and *Parvicrepis* (placed currently within the Diplocrepinae), *Posidonichthys* (Aspasminae), and a number of undescribed taxa from southern Australia (Genus A, B, and C sensu Hutchins, 2008). The placement of *Alabes* with other southern Australian taxa is congruent with the results of Conway et al. (2017a), in which *A. hoesei* was recovered as part of a strongly supported monophyletic group together with *Parvicrepis parvipinnis* and *Cochleoceps orientalis* but not with those of Fricke et al. (2017), who obtained *Alabes* as the sister taxon to a clade comprising the Indo-Pacific taxa *Lepadichthys lineatus* and *Aspasma*

minima. This incongruence likely stems from the limited taxon sampling scheme utilized by Fricke et al. (2017).

4.1c Subfamily Chorisochisminae

The monotypic subfamily Chorisochisminae includes only the South African endemic *Chorisochismus dentex* (Briggs, 1955). Excluding the topologies resulting from the different analyses of the COI and mtDNA datasets and the Bayesian analysis of the ZIC1 and 12S datasets, in the majority of topologies obtained in the present study, *Chorisochismus dentex* is placed in a strongly supported sister group relationship with *Eckloniaichthys scylliorhiniceps*, another South African endemic species (Smith, 1943) that has been previously considered a member of the Gobiesocinae (Briggs, 1955). Conway et al. (2017a) obtained *C. dentex* as the sister taxon to the New World clade. This relationship was not repeated in the topologies obtained herein regardless of dataset or method of analysis. *C. dentex+E. scylliorhiniceps* was obtained in various positions across the resulting topologies of the different analyses. Many of the topologies reported in the present study placed *C. dentex+E. scylliorhiniceps* as the sister group to a clade comprising Old World taxa. However, *C. dentex+E. scylliorhiniceps* was also obtained as the sister group to the lepadogastrine clingfishes in the topologies resulting from the analyses of the nDNA (Appendix C) and ENC1 (Appendix D3 & E3) datasets and as the sister group to all the remaining gobiesocids from the analyses of the MYH6 dataset (Appendix D5 & E5).

4.1d Subfamily Diademichthyinae

Briggs (1955) originally included two genera within the Diademichthyinae, including *Diademichthys* and *Lepadichthys* and later (Briggs, 1976) added *Discotrema*. The genus *Unguitrema* was added subsequently by Fricke (2014). Six species, representing three of the four diademichthyine genera, were available for investigation herein, including: *Diademichthys* (*D. lineatus*), *Discotrema* (*D. crinophila*), and *Lepadichthys* (*L. akiko, L. coccinotaenia, L. frenatus,* and *L. lineatus*).

Regardless of the dataset or method of analysis, these diademichthyine taxa were never obtained as a monophyletic group. Instead, these taxa are consistently obtained as more closely related to members of other subfamilies than to each other, especially members of the Aspasminae and Diplocrepinae, within a larger clade of Indo-Pacific taxa. A small 'core' group of diademichthyine taxa, including *Diademichthys lineatus, Discotrema crinophila,* and *Lepadichthys lineatus,* was recovered as monophyletic in the topologies resulting from the different analyses of the concatenated and individual gene datasets, excluding ENC1, SH3PX3, and ZIC1. The placement of *Lepadichthys lineatus* is contentious (Craig & Randall, 2008) as this species shares a number of external similarities with members of *Discotrema*, including a striking color pattern (white stripes on a red background) and a charismatic obligate association with crinoids (Hayashi & Hayashi, 1985; Hayashi et al., 1986). Though some authors recognize *Lepadichthys lineatus* as a member of *Discotrema* (Eschmeyer, 2006), others (Craig & Randall, 2008) prefer to recognize this species according to its original assignment by Briggs (1976), based on the absence of several characters considered diagnostic for *Discotrema*,

including a deep central cavity in the adhesive disc, no reverse tips on teeth of the upper jaw, no gap between the premaxillae, and the presence of large accessory ossicles posterior to the postcleithrum (Craig & Randall,, 2008). Topologies reported herein include *Lepadichthys lineatus* in a close relationship with *Discotrema crinophila,* not with other members of *Lepadichthys.* These results are congruent with the suspicions of Hayashi & Hayashi (1985) and Hayashi et al. (1986), but a more focused investigation of the Diademichthyinae, including additional members of *Lepadichthys* and *Unguitrema,* will be necessary before solid taxonomic decisions regarding the generic position of *Lepadichthys lineatus* can be made.

In several topologies (e.g., those resulting from the analyses of the combined dataset), *Lepadichthys frenatus* is obtained as the sister taxon to this 'core' group of diademichthyine taxa. This result is similar to that reported by Conway et al. (2017a), in which *L. frenatus* was reported to represent the sister taxon to *Diademichthys lineatus* plus *Discotrema crinophila*. In the topologies obtained from the analyses of the mtDNA dataset, *Lepadichthys frenatus* was obtained in a sister group relationship with *Aspasma minima* (Appendix B), and its placement was variable in the topologies resulting from the analyses of the remaining nuclear gene datasets (Appendix D). The two remaining species of *Lepadichthys* included for analysis were not placed together; *Lepadichthys akiko* was always obtained in a strongly supported relationship with the undescribed "Urchin Clingfish" and *Lepadichthys coccinotaenia* was obtained as the sister taxon to a clade comprised of multiple Indo-Pacific taxa. Based on the topologies obtained herein,

as previously noted by Craig & Randall (2008), the erection of new genera may be necessary to accommodate several of the species currently placed within *Lepadichthys.*

4.1e Subfamily Diplocrepinae

Briggs (1955) originally included seven genera in the subfamily Diplocrepinae, including *Aspasmogaster, Cochleoceps, Diplocrepis, Gastrocyathus, Gastroscyphus, Parvicrepis,* and *Pherallodus* Shiogaki and Dotsu (1983) added *Propherallodus*. Though *Gastrocymba* was originally included as a member of the Trachelochisminae by Briggs (1955), Springer and Fraser (1976) reassigned this taxon to the Diplocrepinae based on the number of observed gill filaments (three versus three and a half). Fourteen species, representing eight of the nine diplocrepine genera, were available for investigation herein, including: *Aspasmogaster* (*A. costata, A. liorhynchus,* and *A. tasmaniensis*), *Cochleoceps* (*C. bassensis, C. orientalis, C. spatula,* and *C. viridis*), *Diplocrepis* (*D. puniceus*), *Gastrocyathus* (*G. gracilis*), *Gastroscyphus* (*G. hectoris*), *Gastrocymba* (*G. quadriradiata*), *Parvicrepis* (*P. parvipinnis* and *P.* sp.), and *Propherallodus* (*P.* sp.).

Diplocrepinae was not obtained as monophyletic regardless of the method of analysis or dataset. Instead, these taxa were consistently obtained as more closely related to members currently assigned to other subfamilies. *Cochleoceps* and *Parvicrepis,* genera endemic to southern Australia, were obtained as part of a larger monophyletic group of southern Australia endemic clingfishes including members of Aspasminae (*Posidonichthys*) and Cheilobranchinae (*Alabes*) and undescribed taxa (Genus A, Genus B, and Genus C), a result congruent with the findings of Conway et al. (2017a) in which

Cochleoceps orientalis, *Pherallodus*, and *Alabes hoesei* were obtained as a strongly supported monophyletic group. *Pherallodus* and *Propherallodus,* genera endemic to the Indo-Pacific, were obtained as part of a larger monophyletic group of Indo-Pacific endemic clingfishes including members of the Aspasminae (*Aspasmichthys*) and Diademichthyinae (*Diademichthys, Discotrema,* and *Lepadichthys*) and the undescribed "Kermadec Clingfish" and "Urchin Clingfish". *Aspasmogaster,* endemic to southern Australia, was obtained as the sister group to this Indo-Pacific clade, while the New Zealand endemics *Gastrocyathus, Gastroscyphus,* and *Gastrocymba* were obtained with another New Zealand endemic, *Haplocylix,* a member of Haplocylicinae. *Diplocrepis,* another New Zealand endemic, and the type genus of the Diplocrepinae, was obtained as the sister taxon to all remaining gobiesocids in the topologies resulting from the analyses of the nDNA and combined datasets but was variable in its position across the individual gene tree topologies.

4.1f Subfamily Gobiesocinae

Briggs (1955) originally included *Acyrtops, Acyrtus, Arcos, Eckloniaichthys, Gobiesox, Pherallodiscus* (recently synonymized with *Gobiesox* by Conway et al., 2017a)*, Rimicola, Sicyases,* and *Tomicodon* in his Gobiesocinae, and later (Briggs, 1969) added *Derilissus.* Twenty-nine described and two undescribed species, representing all nine genera, were available for investigation herein, including: *Acyrtops* (*A. beryllinus*), *Acyrtus* (*A. artius, A. lanthanum*, and *A. rubiginosus*), *Arcos* (*A. erythrops*), *Derilissus* (*D.* sp.), *Eckloniaichthys* (*E. scylliorhiniceps*), *Gobiesox* (*G. adustus, G. barbatulus, G.*

cephalus, G. daedelus, G. funebris, G. juradoensis, G. maeandricus, G. mexicanus, G. pinniger, G. potamius, G. punctulatus, G. nigripinnis, G. rhessodon, and *G. strumosus*), *Rimicola* (*R. muscarum*), *Sicyases* (*S. sanguineus*), and *Tomicodon* (*T. boehlkei, T. briggsi, T. humeralis, T. lavettsmithi, T. myersi, T. reitzae, T.* sp. and *T. zebra*).

Gobiesocinae (sensu Briggs, 1955) was not obtained as monophyletic regardless of the method of analysis or dataset. Though all New World members of the Gobiesocinae were obtained as a monophyletic group, the South African endemic *Eckloniaichthys scylliorhiniceps* was consistently placed in a strongly supported sister group relationship with *Chorisochismus dentex*, another South African endemic species. Briggs (1955) considered *Eckloniaichthys* to be closely related to *Rimicola* and *Acyrtops,* based on similarities of the adhesive disc, a narrow upper lip, and the length of the lower pectoral-fin rays. A recent study by Conway et al. (2015) reported striking similarities between the oral dentition of *Eckloniaichthys* and several Indo-Pacific taxa belonging to the Diademichthyinae, Aspasminae, and Diplocrepinae, raising suspicion about the placement of *Eckloniaichthys* with the New World taxa. The results presented herein support the suspicions of Conway et al. (2015) that the closest living relative of *E. scylliorhiniceps* is not among the New World taxa but do not support the suspected close relationship of this species and Indo-Pacific taxa.

All remaining members of the Gobiesocinae (the New World taxa) were obtained as a strongly supported monophyletic group in the topologies resulting from the analyses of the combined and nDNA datasets. These results are consistent with the majority of previous molecular phylogenetic investigations of the Gobiesocidae that have obtained a

monophyletic group of New World taxa (Fricke et al., 2017; Conway et al., 2014, 2017a). The recovery of a clade of New World clingfishes in the recent molecular phylogenetic studies is congruent with the results of a recent morphology-based study (Conway et al., 2017a) which proposed that the New World taxa formed a monophyletic group based on putative morphological synapomorphies (derived from features of oral dentition).

The intrarelationships of the New World gobiesocids presented herein conflict with the results obtained by previous studies. For example, *Rimicola* + *Acyrtops* was obtained by Conway et al. (2017a) as the sister group to *Tomicodon*, but instead this clade is placed as the sister group to *Derilissus* in the topologies resulting from the different analyses of the combined (Fig. 1 & Appendix A), nDNA (Appendix C), and SH3PX3 (Appendix D6 & E6) datasets. *Acyrtus*+*Arcos* were obtained as the sister group to *Gobiesox* in the phylogenetic hypotheses presented by Conway et al. (2014) and Conway et al. (2017a). While this relationship is present in the topologies recovered from the Bayesian analyses of the combined and nDNA datasets obtained herein, the topologies resulting from the ML analyses of the same datasets include *Acyrtus*+*Arcos* as the sister taxon of *Tomicodon,* a result congruent with the findings of Fricke et al. (2017).

4.1g Subfamily Haplocylicinae

Briggs (1955) originally included *Haplocylix* as the only genus of the Haplocylicinae. Böhlke and Robins (1970) added *Gymnoscyphus*. Of the two monotypic genera, only one species, *Haplocylix littoreus*, was available for investigation. The topologies

resulting from the analyses of the combined, nDNA, GLYT, and SH3PX3 datasets and the species tree obtained *Haplocylix littoreus* in a clade together with *Gastroscyphus hectoris, Gastrocyathus gracilis*, and *Gastrocymba quadriradiata,* all currently assigned to the Diplocrepinae. Resulting topologies from the analysis of the COI and mtDNA datasets obtained *Haplocylix littoreus* as the sister taxon to *Eckloniaichthys scylliorhiniceps,* a member of the Gobiesocinae. The diplocrepines mentioned plus *Haplocylix littoreus* are all endemic to New Zealand, however, *Eckloniaichthys scylliorhiniceps* is endemic to South Africa. Even though there is controversy in which taxon is the closest relative of *Haplocylix, Haplocylix littoreus* was always recovered as the sister taxon to or in a polytomy with a clade comprising southern Australia, Indo Pacific, and New Zealand endemic clingfishes. This is the first time that the phylogenetic position of *Haplocylix littoreus* has been analyzed in regards to other gobiesocids.

4.1h Subfamily Lepadogastrinae

Briggs (1955) originally included five genera within the Lepadogastrinae, including, *Apletodon, Diplecogaster, Gouania, Lepadogaster,* and *Opeatogenys* and later (Briggs, 1957) added *Lecanogaster.* Eight species, representing five of the six lepadogastrine genera, were available for investigation herein, including: *Apletodon* (*A. dentatus* and *A. incognitus*)*, Diplecogaster* (*D. bimaculata*), *Gouania* (*G. willdenowi*)*, Lepadogasater* (*L. candollei, L. Lepadogaster,* and *L. purpurea*) and *Opeatogenys* (*O. gracilis*).

The eastern Atlantic lepadogastrine clingfishes were consistently obtained in a strongly supported monophyletic group in all topologies resulting from the different analyses except for the COI and 12S sequence data which obtained *Apletodon* in a relationship with *Arcos erythrops*, a member of the Gobiesocinae. *Opeatogenys gracilis* was obtained as part of the outgroup in the topology resulting from the ML analysis of the COI dataset. Congruent with the current study, Fricke et al. (2017) also obtained Lepadogastrinae as a monophyletic group (represented by *Lepadogaster* and *Apletodon*). However, using 12S and 16S sequence data, Almada et al. (2008) did not obtain Lepadogastrinae as monophyletic because of a relationship obtained between *Aspasma minima,* a member of the Aspasminae, and *Lepadogaster candollei*. This relationship was not recovered in any topologies presented herein. All topologies resulting from the analyses of the included datasets, except the MYH6 and mtDNA datasets, obtained *Diplecogaster bimaculata+Opeatogenys gracilis* as the sister group to all included members of the Lepadogastrinae. Almada et al. (2008) obtained members of *Apletodon* as the sister group to the remaining members of Lepadogastrinae, a relationship only recovered in the topology resulting from the analyses of the MYH6 dataset presented herein. *Gouania willdenowi* was obtained as the sister taxon to *Lepadogaster lepadogaster* and *Lepadogasater purpurea* which is congruent with Almada et al. (2008). However, these aforementioned taxa were placed as the sister group to a clade comprising *Apletodon dentatus, Apletodon incognitus,* and *Lepadogaster candollei* which is not congruent with the findings of Almada et al. (2008). The placement of Lepadogastrinae in regards to the remaining members of the Gobiesocidae varies across

datasets and method of analysis. The topologies resulting from the ML analysis of the nDNA and combined datasets obtained the Lepadogastrinae as the sister group to all remaining gobiesocids, excluding *Diplocrepis puniceus.* The topologies resulting from the Bayesian analysis obtained the Lepadogastrinae in a polytomy with the remaining gobiesocids (excluding *D. puniceus*) for the combined dataset and as the sister group to the South African endemic *Chorisochismus dentex* and *Eckloniaichthys scylliorhiniceps* for the nDNA dataset. Despite this conflict, the monophyly of the Lepadogastrinae is well supported.

4.1i Subfamily Protogobiesocinae

Fricke et al. (2017) erected Protogobiesocinae for *Lepadicyathus mendeleevi* Prokofiev (2005) and the newly discovered *Protogobiesox asymmetricus* (Fricke et al., 2017). Only mtDNA sequences of *Protogobiesox asymmetricus* were available for analysis herein.

Fricke et al. (2017) obtained *Protogobiesox asymmetricus* as the sister taxon to members of the Aspasminae, Diademichthyinae, and Cheilobranchinae. Fricke et al. (2017) suggested that the members of the Protogobiesocinae were morphologically most similar to members of the Aspasminae. Despite this suspected morphological similarity, a close relationship was not recovered herein between the members of the Aspasminae and Protogobiesocinae regardless of dataset or method of analysis. With the large addition of taxa included in this study, topologies resulting from the analyses of the COI and mtDNA datasets revealed a relationship between *Protogobiesox asymmetricus* and *Gastrocymba quadriradiata,* a member of the Diplocrepinae. Topologies resulting from

the analyses of the 12S dataset obtained *Protogobiesox asymmetricus* as the sister taxon to the Gobiesocinae and Lepadogastrinae for the ML analyses and as part of a polytomy containing the same aforementioned taxa for the Bayesian analysis.

4.1j Subfamily Trachelochisminae

Briggs (1955) originally included four genera within the Trachelochisminae, including *Conidens, Creocele, Dellichthys,* and *Trachelochismus.* Hardy (1984) added *Kopua*. Six described and one undescribed species, representing four of the five genera, were available for investigation herein, including *Conidens* (*C. laticephalus*), *Creocele* (*C. cardinalis*), *Dellichthys* (*D. morelandi* and *D.* sp.), and *Trachelochismus* (*T. aestuarium, T. pinnulatus,* and *T. melobesia*).

Trachelochisminae was obtained as monophyletic only in the topologies resulting from the analyses of the nDNA dataset and the ML analysis of the combined dataset where Trachelochisminae was placed as the sister group to a clade comprising members of Aspasminae (*Aspasmichthys*), Diademichthyinae (*Diademichthys, Discotrema,* and *Lepadichthys*), Diplocrepinae (*Aspasmogaster, Pherallodus,* and *Propherallodus*) and the undescribed "Urchin Clingfish". *Trachelochismus melobesia* was obtained as the sister taxon to *Trachelochismus aestuarium* and *Trachelochismus pinnulatus* in every recovered topology except for the SH3PX3 gene trees that recovered members of *Trachelochismus* in a polytomy and the ZIC1 gene trees that placed *Trachelochismus aestuarium* as the sister taxon to *Trachelochismus melobesia* and *Trachelochismus pinnulatus.* And with the inclusion of additional nDNA genes, a phylogenetic

relationship is supported more often in the topologies presented herein between *Trachelochismus aestuarium* and *Trachelochismus pinnulatus.*

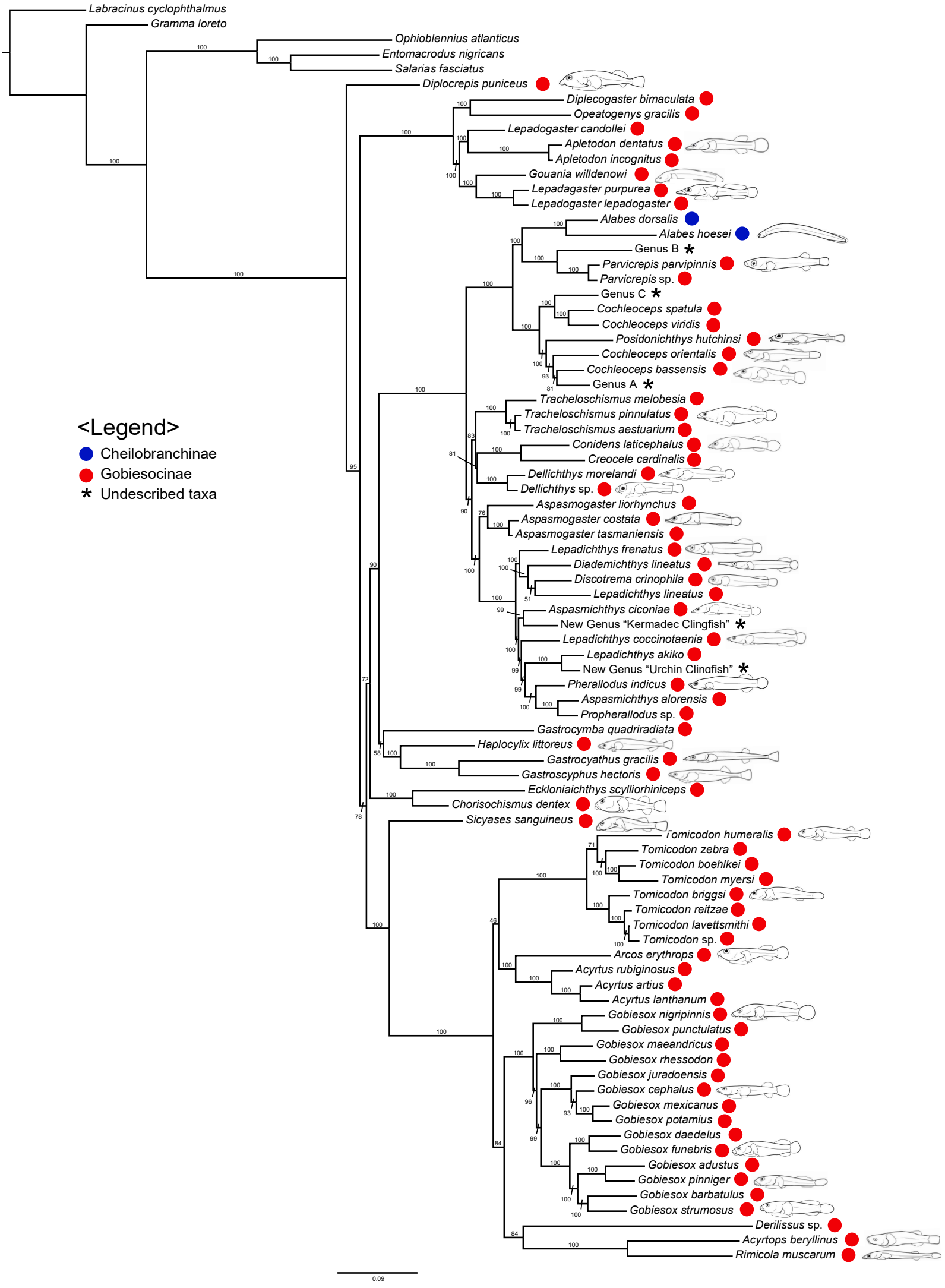
Dellichthys morelandi and *Dellichthys* sp. were always obtained as a monophyletic group, however, the position of *Dellichthys* varied among the recovered topologies. *Dellichthys* was obtained as the sister group to *Conidens laticephalus* and *Creocele cardinalis* as part of a clade containing the remaining members of Trachelochisminae in the topologies resulting from the analyses of the nDNA dataset and the ML analysis of the combined dataset. However, the recovered topology from the Bayesian analysis of the combined dataset placed *Dellichthys* as the sister group to *Trachelochismus. Conidens laticephalus*+*Creocele cardinalis* were obtained as the sister group to a group of Indo-Pacific clingfishes comprising members of Aspasminae (*Aspasmichthys*), Diademichthyinae (*Diademichthys, Discotrema,* and *Lepadichthys*), Diplocrepinae (*Aspasmogaster, Pherallodus,* and *Propherallodus*) and the undescribed "Urchin Clingfish" in the topology resulting from the Bayesian analysis of the combined dataset. *Dellichthys* and *Trachelochismus* are both endemic to New Zealand, *Creocele* is endemic to southern Australia, and *Conidens* is widespread throughout the tropical Indo-Pacific region (Briggs, 1955).

While the position of each of the trachelochismine genera is variable across the recovered topologies, these taxa are always obtained as close relatives of the clade of southern Australian endemic clingfishes and the Indo-Pacific clade comprising members of the Aspasminae, Diademichthyinae and the Diplocrepinae.

4.2 Remarks on the two alternative classifications

The alternative classification scheme utilized by Van der Laan et al. (2014) divides the gobiesocids between the Cheilobranchinae (including *Alabes* only) and the Gobiesocinae including all remaining genera) (Fig. 2). This alternative classification scheme was potentially established to account for the vast number of osteological differences observed between *Alabes* and other gobiesocids, most notably the absence of an adhesive disc in *Alabes* (Springer & Fraser, 1976). The results obtained here from the analyses of nDNA and mtDNA sequence data obtained *Alabes* as part of a larger monophyletic group comprised exclusively of clingfishes endemic to southern Australia, including species currently assigned to the Diplocrepinae (*Cochleoceps* and *Parvicrepis*), Aspasminae (*Posidonichthys*), and the undescribed Genus A, Genus B, and Genus C. The results presented herein are congruent with recent studies on the investigation of the Gobiesocidae that obtained *Alabes* deeply embedded within the Gobiesocidae and not as the sister taxon to the remaining members of the Gobiesocidae (Conway et al., 2017a; Fricke et al., 2017). Furthermore, the alternative two subfamily classification and the classification scheme established by Briggs (1955) (Fig. 1) were significantly different from the topology resulting from the analysis of the combined dataset (excluding the 5 undescribed taxa) when compared using likelihood ratio tests (Table 4). This provides additional support that the two available classification schemes do not accurately reflect the evolutionary relationships of the Gobiesocidae.

Figure 2. Phylogram obtained from the Maximum Likelihood analysis of the combined dataset labeled according to the two subfamilies in the classification scheme utilized by Van der Laan et al. (2014) and Nelson et al. (2016). Numbers above branches represent bootstrap support values.



5. CONCLUSION

Presented herein is the first large scale multi-locus investigation on the phylogenetic relationships of the Gobiesocidae. All topologies obtained from the analyses conducted herein revealed that there are significant problems associated with Briggs' (1955) morphology-based classification scheme. There are four subfamilies (Aspasminae, Diademichthyinae, Diplocrepinae, and Gobiesocinae) and four genera (*Aspasmichthys, Cochleoceps, Lepadichthys,* and *Lepadogaster*) that are potentially not monophyletic. These results are in line with earlier criticisms of Briggs (1955) classification scheme and the need for revision of this system. Furthermore, *Alabes* was obtained as deeply embedded within the Gobiesocidae signifying that the alternative classification scheme advocated by Van der Laan et al. (2014) and Nelson et al. (2016) also does not accurately reflect the evolutionary relationships of the group. The results presented herein will provide future researchers working with these fishes with a taxonomic roadmap for classifying newly discovered species as well as a phylogenetic framework for investigating the many interesting anatomical and ecological characters of clingfishes.

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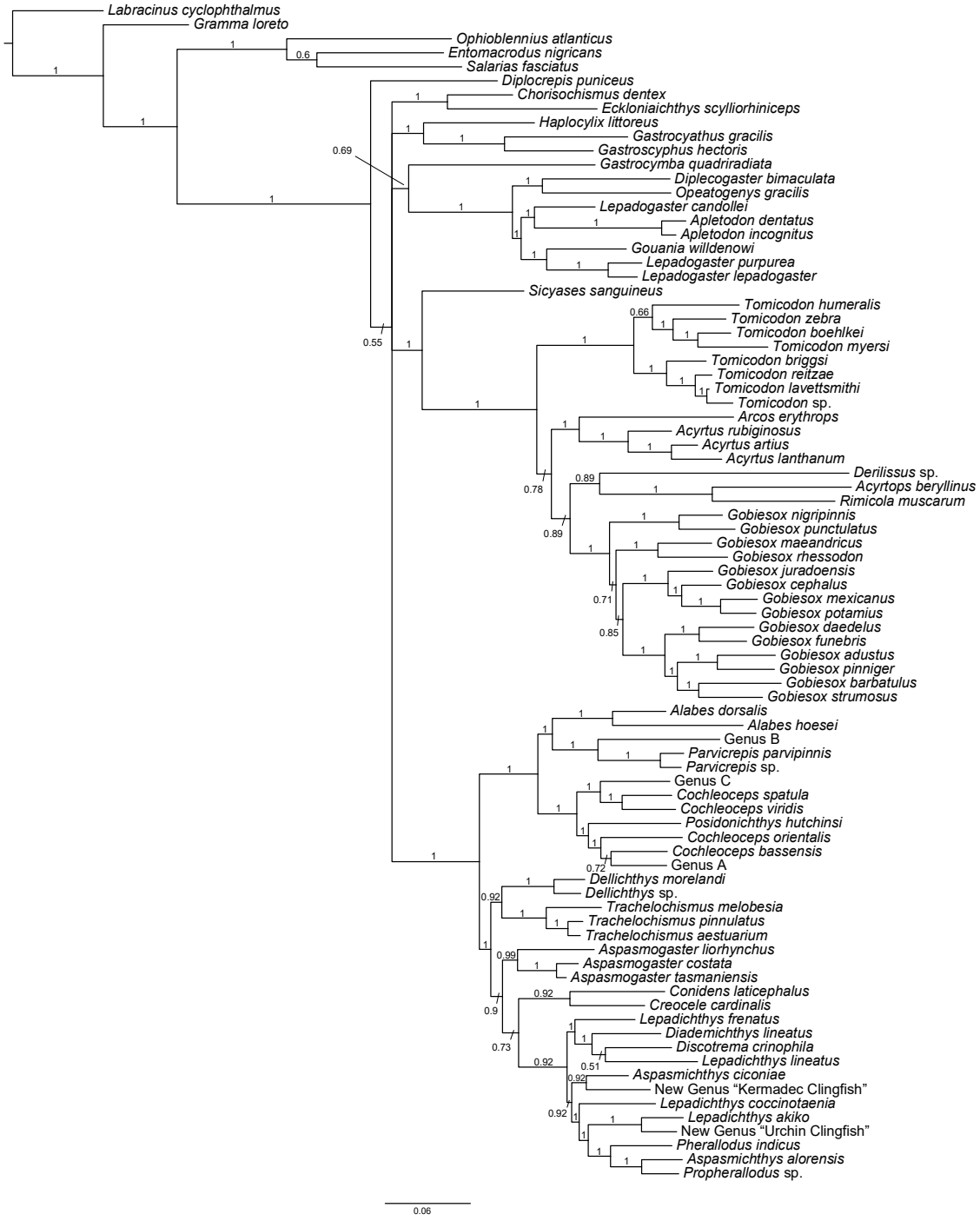
APPENDIX A

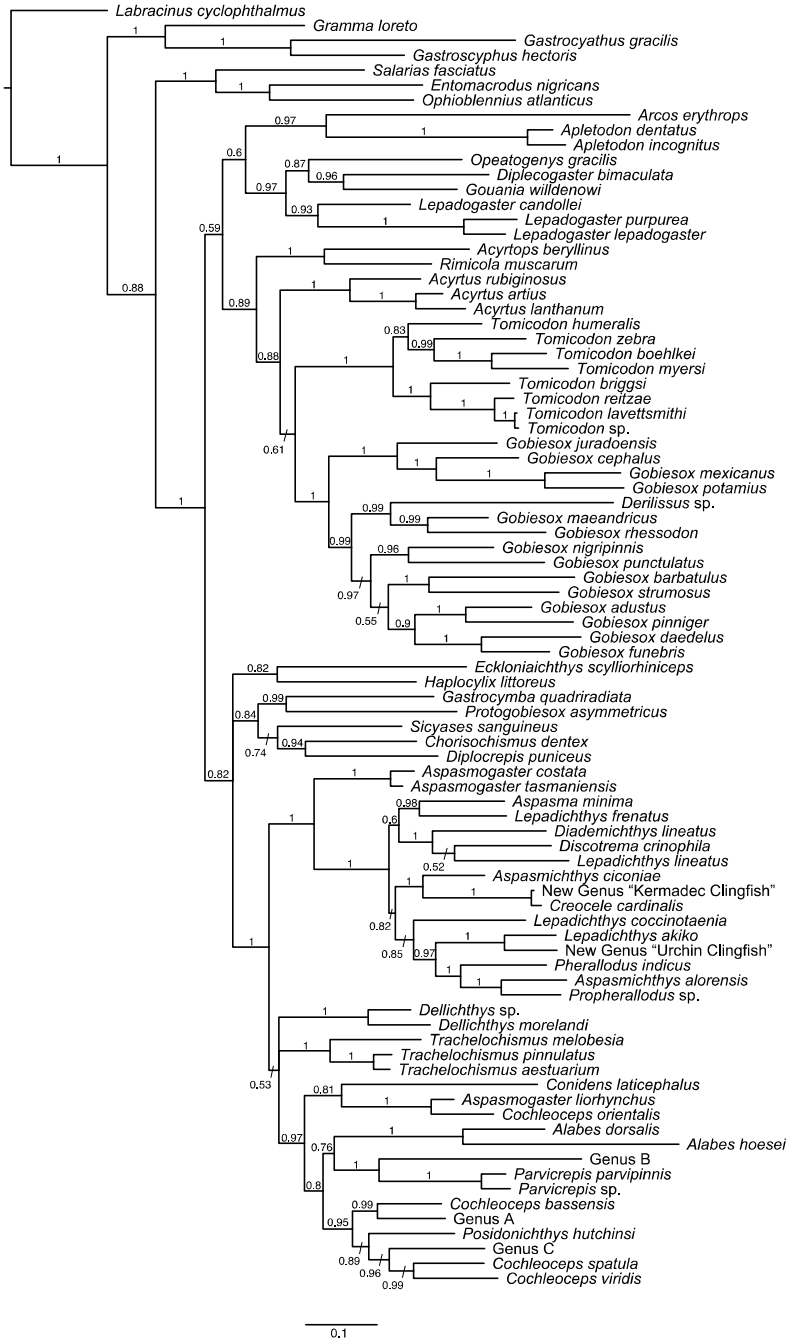
Phylogram obtained from the Bayesian analysis of the combined dataset. Numbers above branches represent posterior probabilities.



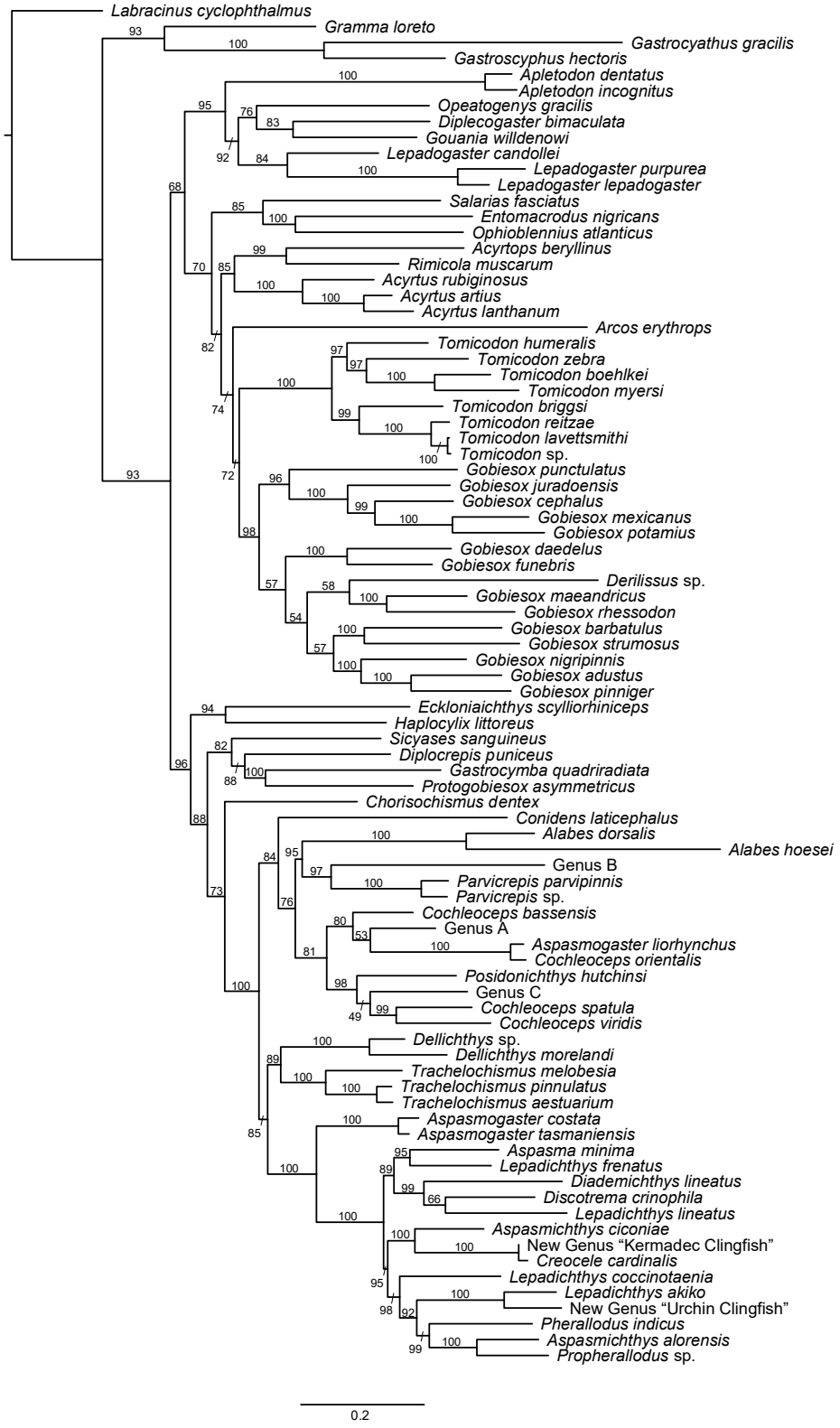
APPENDIX B

Topologies resulting from the analysis of the mtDNA dataset. (1) Bayesian phylogram. (2) Maximum Likelihood phylogram. Numbers above branches represent posterior probabilities (1) or bootstrap values (2).

1. mtDNA - Bayesian



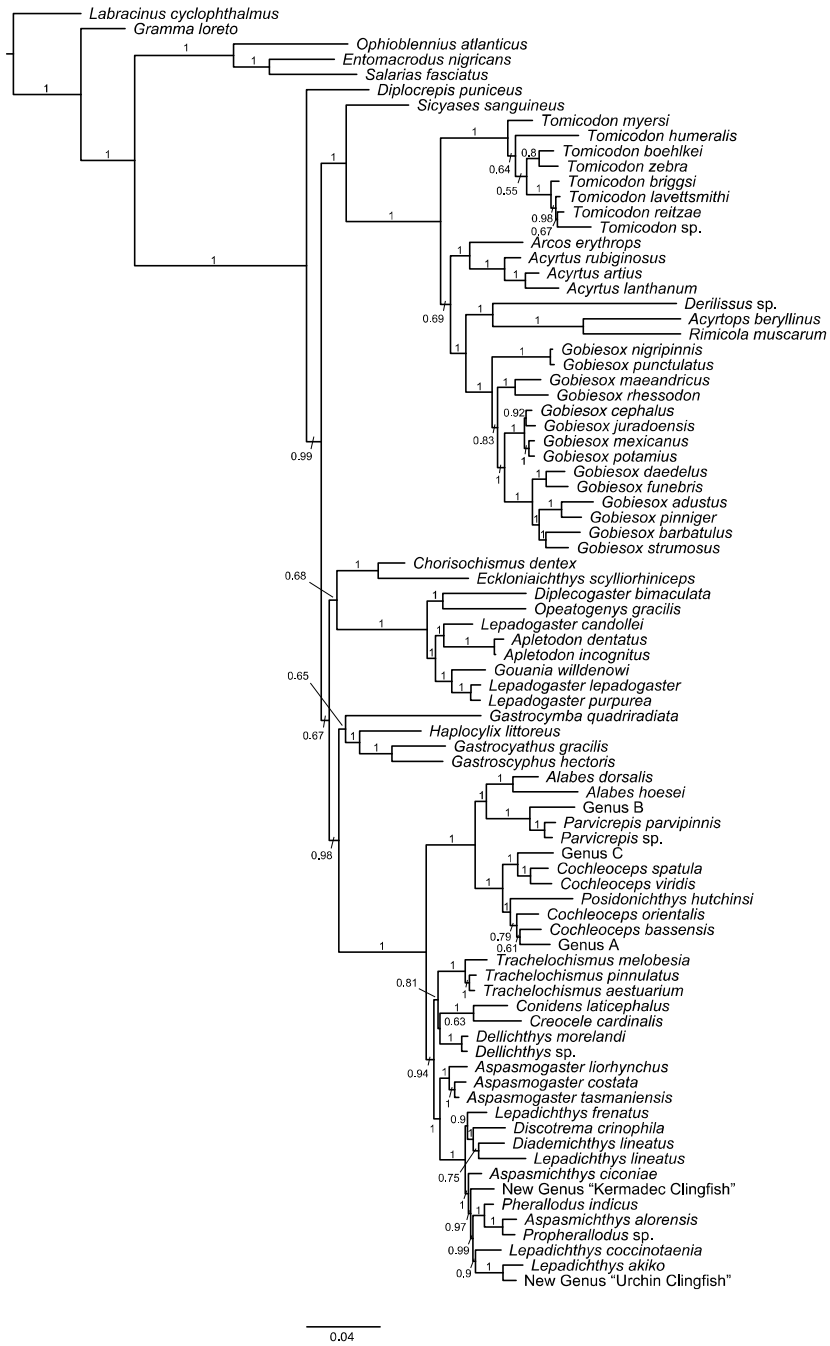
2. mtDNA - Maximum Likelihood



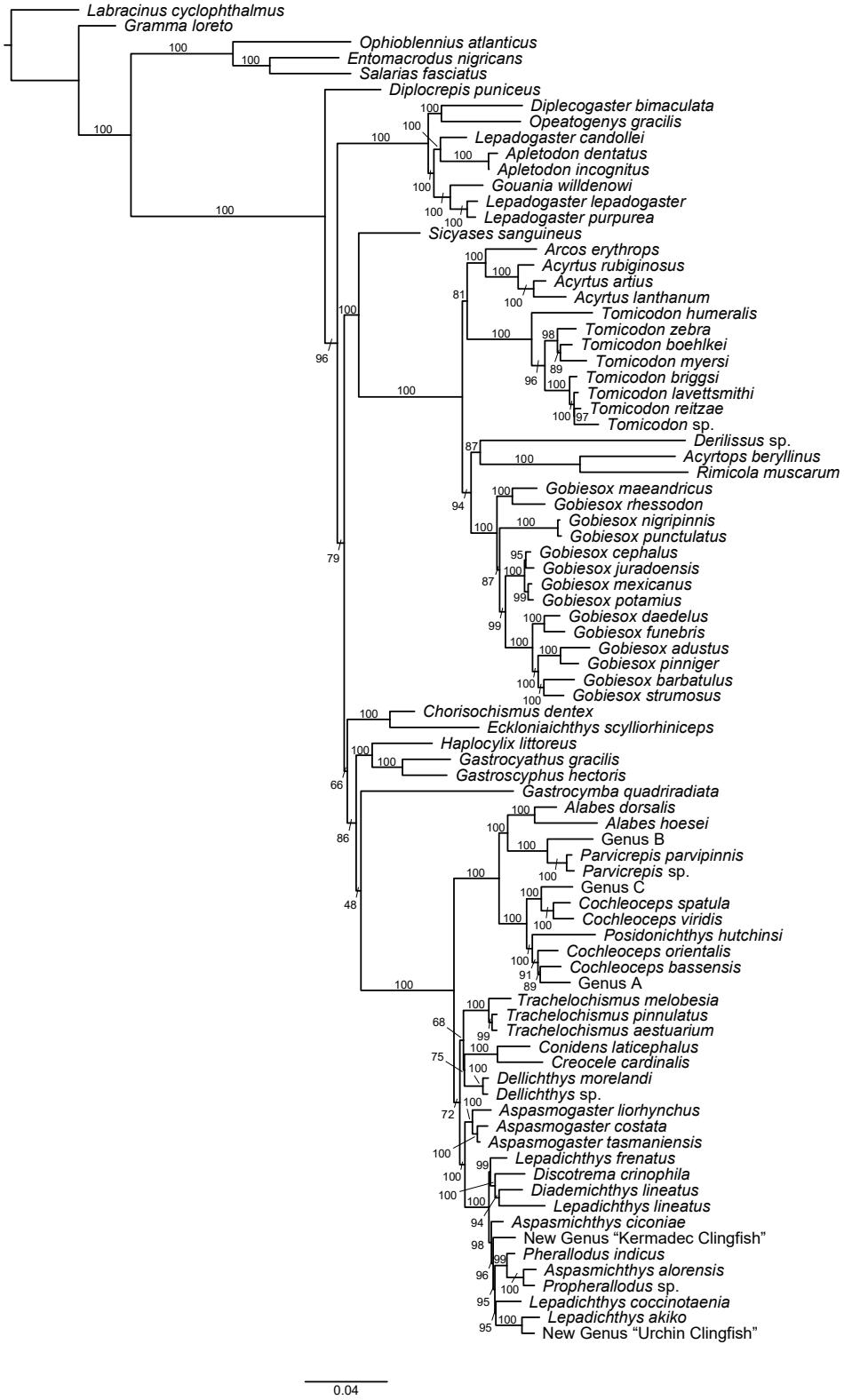
APPENDIX C

Topologies resulting from the analysis of the nDNA dataset. (1) Bayesian phylogram. (2) Maximum Likelihood phylogram. Numbers above branches represent posterior probabilities (1) or bootstrap values (2).

1. nDNA - Bayesian



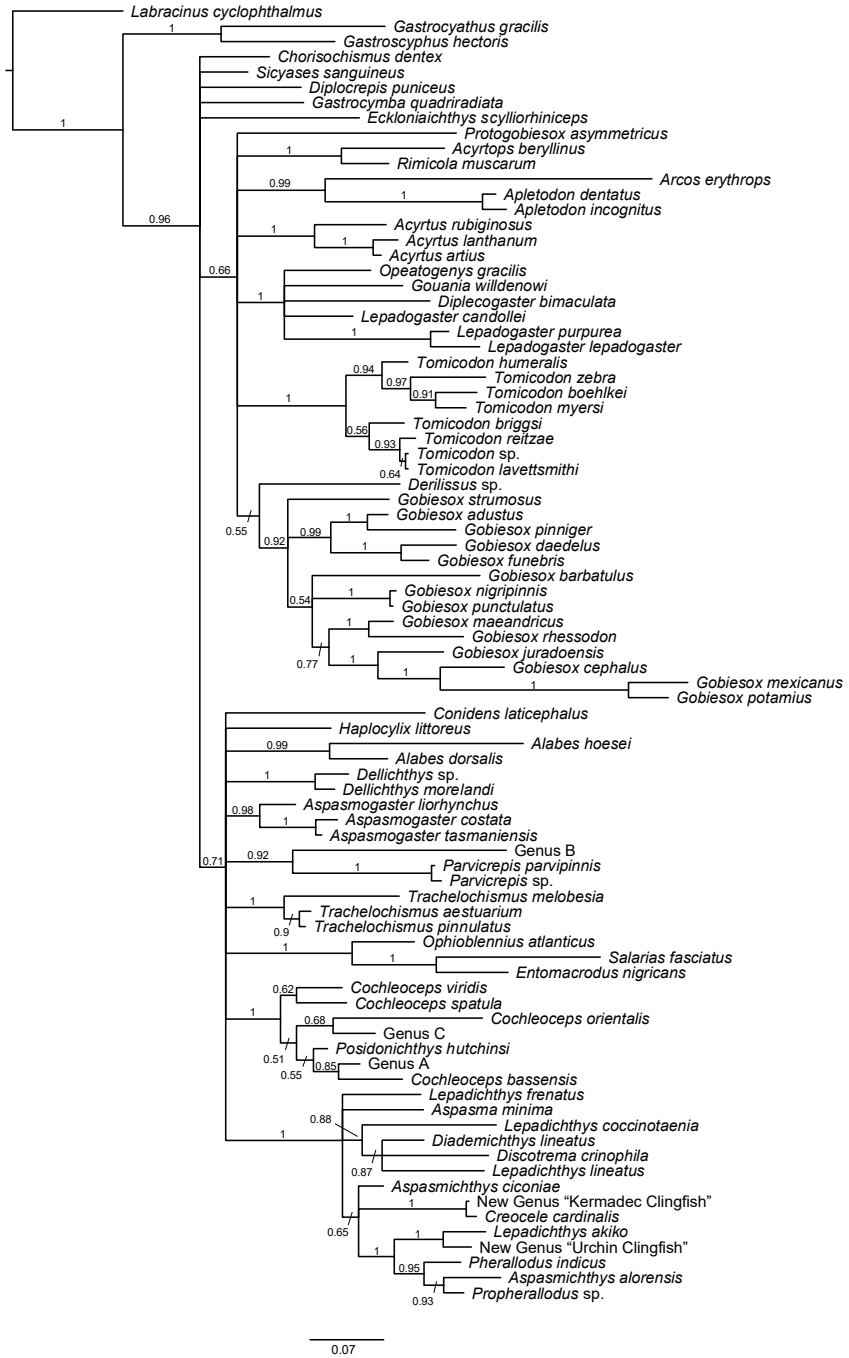
2. nDNA - Maximum Likelihood



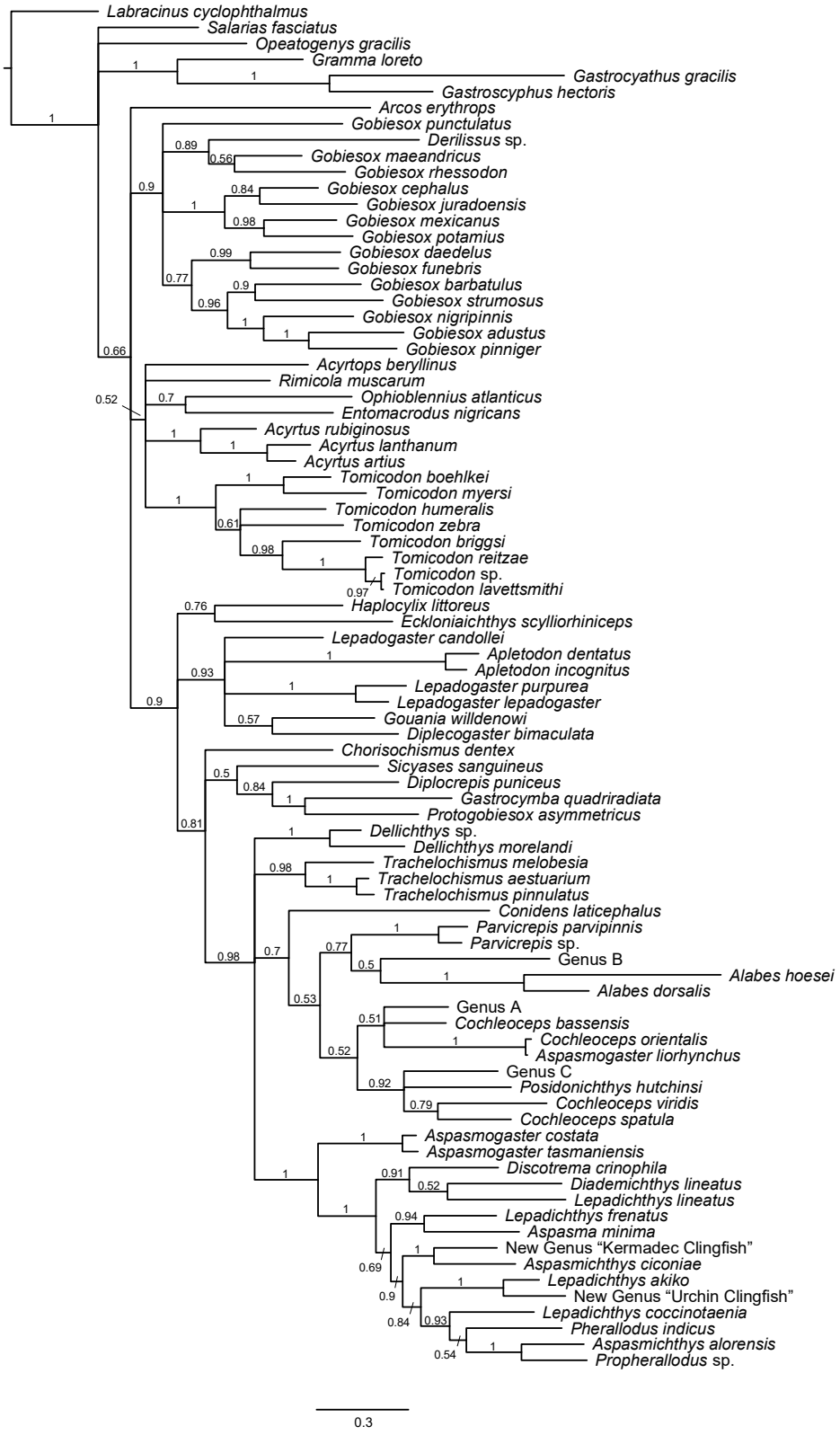
APPENDIX D

Phylograms obtained from the Bayesian analyses of the 12S (1), COI (2), ENC1 (3), GLYT (4), MYH6 (5), SH3PX3 (6), and ZIC1 (7) datasets. Numbers above branches represent posterior probabilities.

1. 12S



2. COI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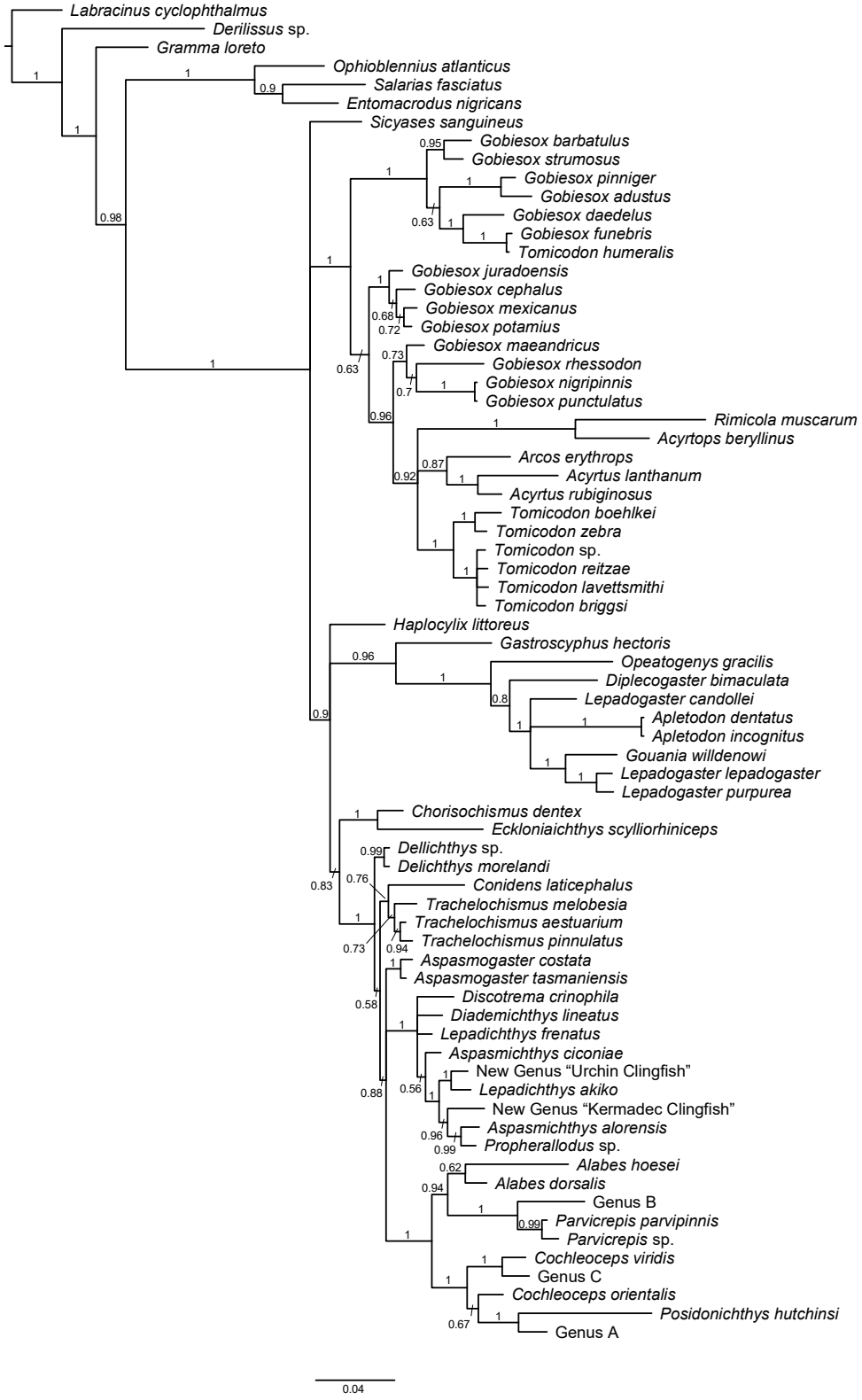
Salarias fasciatus
Opeatogenys gracilis
Gramma loreto
Gastrocyathus gracilis
Gastroscyphus hectoris
Arcos erythrops
Gobiesox punctulatus
Derilissus sp.
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox daedelus
Gobiesox funebris
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox nigripinnis
Gobiesox adustus
Gobiesox pinniger
Acyrtops beryllinus
Rimicola muscarum
Ophioblennius atlanticus
Entomacrodus nigricans
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Tomicodon boehlkei
Tomicodon myersi
Tomicodon humeralis
Tomicodon zebra
Tomicodon briggsi
Tomicodon reitzae
Tomicodon sp.
Tomicodon lavettsmithi
Haplocylix littoreus
Eckloniaichthys scylliorhiniceps
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Lepadogaster purpurea
Lepadogaster lepadogaster
Gouania willdenowi
Diplecogaster bimaculata
Chorisochismus dentex
Sicyases sanguineus
Diplocrepis puniceus
Gastrocymba quadriradiata
Protogobiesox asymmetricus
Dellichthys sp.
Dellichthys morelandi
Trachelochismus melobesia
Trachelochismus aestuarium
Trachelochismus pinnulatus
Conidens laticephalus
Parvicrepis parvipinnis
Parvicrepis sp.
Genus B
Alabes hoesei
Alabes dorsalis
Genus A
Cochleoceps bassensis
Cochleoceps orientalis
Aspasmogaster liorhynchus
Genus C
Posidonichthys hutchinsi
Cochleoceps viridis
Cochleoceps spatula
Aspasmogaster costata
Aspasmogaster tasmaniensis
Discotrema crinophila
Diademichthys lineatus
Lepadichthys lineatus
Lepadichthys frenatus
Aspasma minima
New Genus “Kermadec Clingfish”
Aspasmichthys ciconiae
Lepadichthys akiko
New Genus “Urchin Clingfish”
Lepadichthys coccinotaenia
Pherallodus indicus
Aspasmichthys alorensis
Propherallodus sp.
0.3

3. ENC1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Gramma loreto
Ophioblennius atlanticus
Salarias fasciatus
Entomacrodus nigricans
Sicyases sanguineus
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox pinniger
Gobiesox adustus
Gobiesox daedelus
Gobiesox funebris
Tomicodon humeralis
Gobiesox juradoensis
Gobiesox cephalus
Gobiesox mexicanus
Gobiesox potamius
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox nigripinnis
Gobiesox punctulatus
Rimicola muscarum
Acyrtops beryllinus
Arcos erythrops
Acyrtus lanthanum
Acyrtus rubiginosus
Tomicodon boehlkei
Tomicodon zebra
Tomicodon sp.
Tomicodon reitzae
Tomicodon lavettsmithi
Tomicodon briggsi
Haplocylix littoreus
Gastroscyphus hectoris
Opeatogenys gracilis
Diplecogaster bimaculata
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Gouania willdenowi
Lepadogaster lepadogaster
Lepadogaster purpurea
Chorisochismus dentex
Eckloniaichthys scylliorhiniceps
Dellichthys sp.
Delichthys morelandi
Conidens laticephalus
Trachelochismus melobesia
Trachelochismus aestuarium
Trachelochismus pinnulatus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Discotrema crinophila
Diademichthys lineatus
Lepadichthys frenatus
Aspasmichthys ciconiae
New Genus “Urchin Clingfish”
Lepadichthys akiko
New Genus “Kermadec Clingfish”
Aspasmichthys alorensis
Propherallodus sp.
Alabes hoesei
Alabes dorsalis
Genus B
Parvicrepis parvipinnis
Parvicrepis sp.
Cochleoceps viridis
Genus C
Cochleoceps orientalis
Posidonichthys hutchinsi
Genus A
0.04

4. GLYT


Labracinus cyclophthalmus
Gramma loreto
Salarias fasciatus
Ophioblennius atlanticus
Genus B
Alabes hoesei
Alabes dorsalis
Cochleoceps orientalis
Posidonichthys hutchinsi
Genus A
Genus C
Cochleoceps viridis
Cochleoceps spatula
Creocele cardinalis
Conidens laticephalus
Dellichthys morelandi
Dellichthys sp.
Trachelochismus melobesia
Trachelochismus pinnulatus
Tracheloschismus aestuarium
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Lepadichthys frenatus
Discotrema crinophila
Diademichthys lineatus
Lepadichthys lineatus
New Genus "Kermadec Clingfish"
Aspasmichthys ciconiae
Propherallodus sp.
Aspasmichthys alorensis
Lepadichthys coccinotaenia
New Genus "Urchin Clingfish"
Lepadichthys akiko
Diplecogaster bimaculata
Opeatogenys gracilis
Lepadogaster purpurea
Lepadogaster lepadogaster
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Diplocrepis puniceus
Eckloniaichthys scylliorhiniceps
Chorisochismus dentex
Gastrocymba quadriradiata
Haplocylix littoreus
Gastrocyathus gracilis
Gastroscyphus hectoris
Sicyases sanguineus
Arcos erythrops
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Tomicodon zebra
Tomicodon boehlkei
Tomicodon briggsi
Tomicodon lavettsmithi
Tomicodon reitzae
Acyrtops beryllinus
Rimicola muscarum
Derilissus sp.
Gobiesox rhessodon
Gobiesox maeandricus
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox punctulatus
Gobiesox funebris
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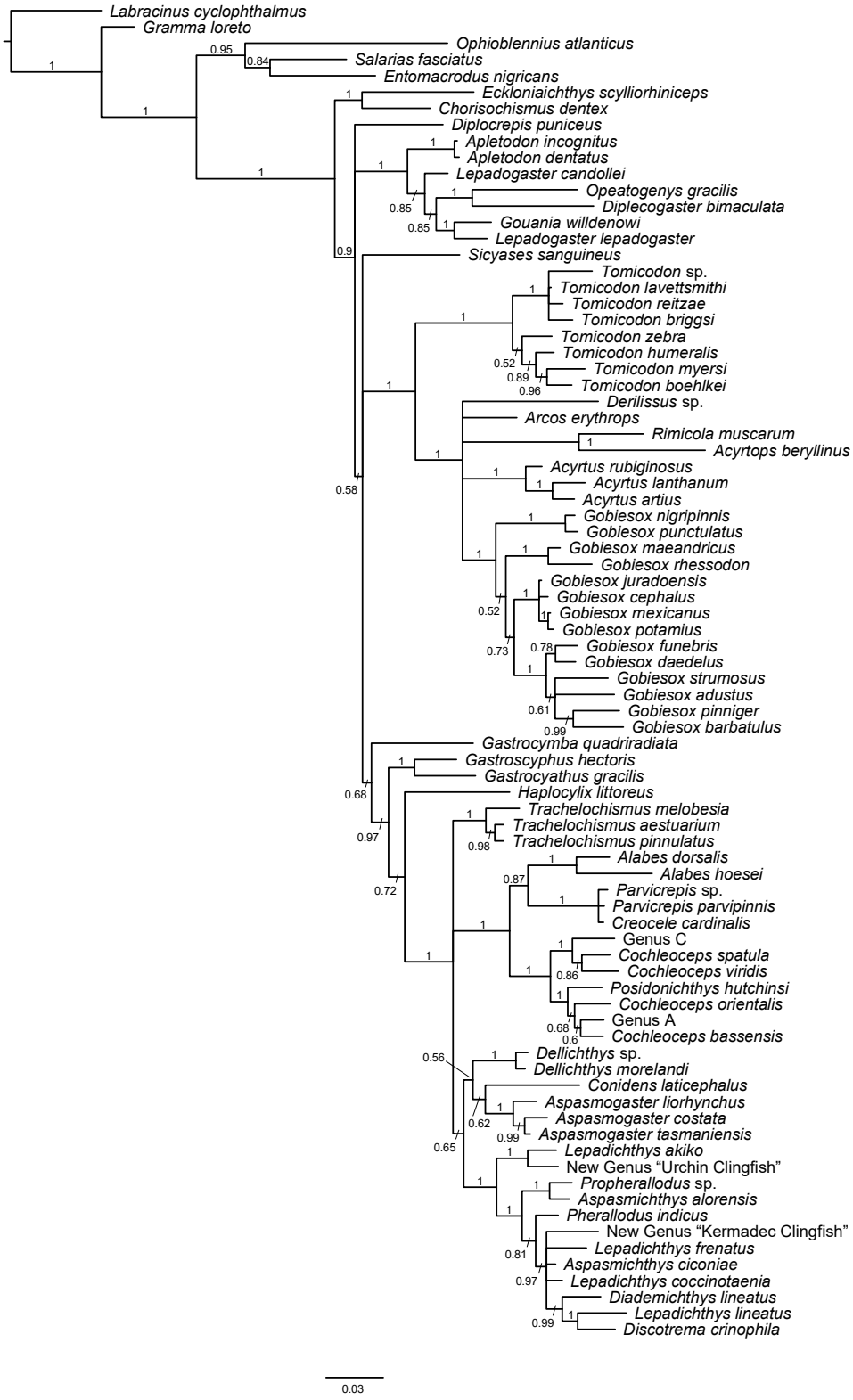
Gobiesox pinniger
Gobiesox barbatulus
Gobiesox strumosus
0.04

5. MYH6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Gastrocymba quadriradiata
Gastroscyphus hectoris
Gastrocyathus gracilis
Haplocylix littoreus
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Trachelochismus aestuarium
Trachelochismus pinnulatus
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Alabes hoesei
Parvicrepis sp.
Parvicrepis parvipinnis
Creocele cardinalis
Genus C
Cochleoceps spatula
Cochleoceps viridis
Posidonichthys hutchinsi
Cochleoceps orientalis
Genus A
Cochleoceps bassensis
Dellichthys sp.
Dellichthys morelandi
Conidens laticephalus
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
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Aspasmichthys alorensis
Pherallodus indicus
New Genus “Kermadec Clingfish”
Lepadichthys frenatus
Aspasmichthys ciconiae
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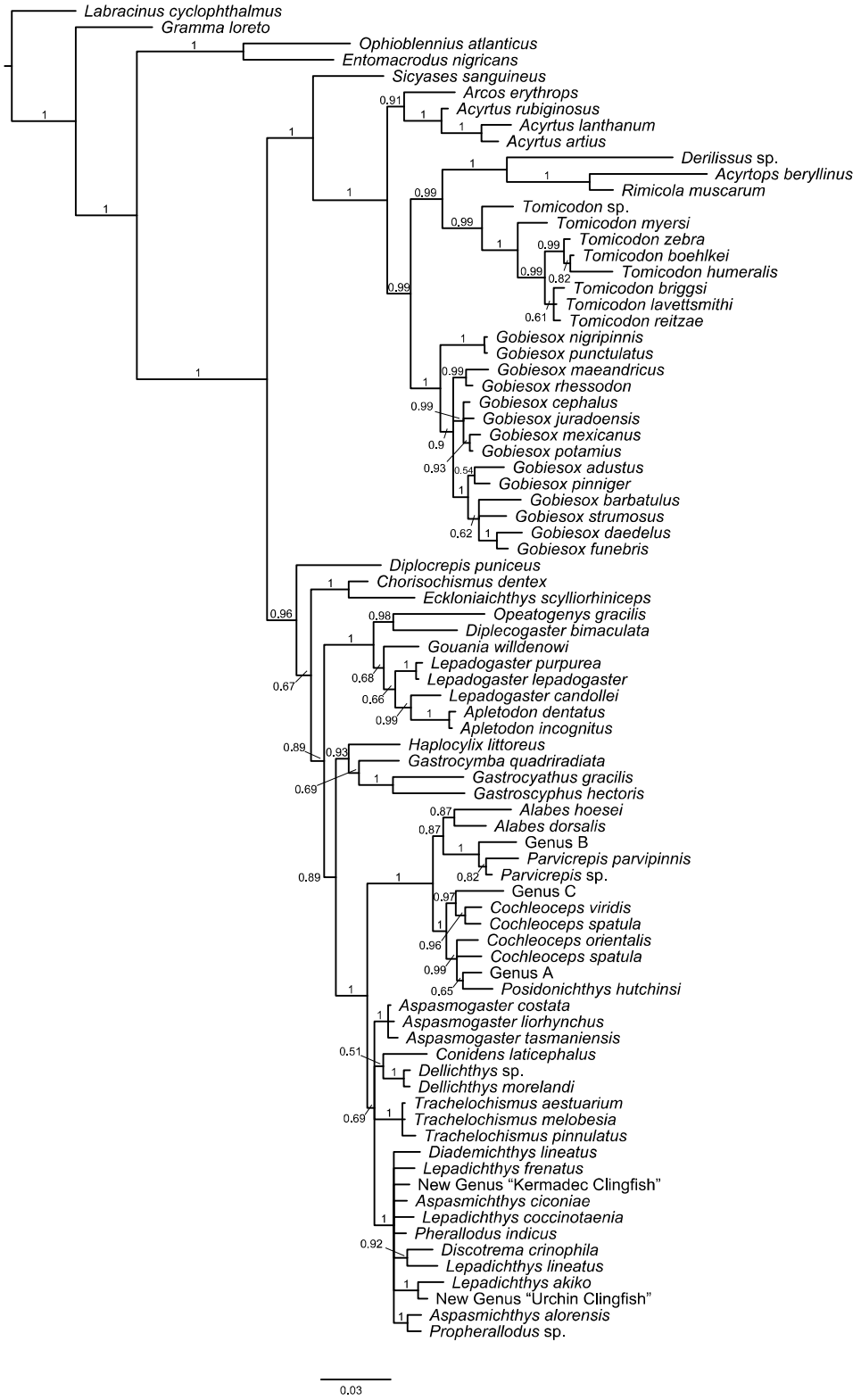
Discotrema crinophila
0.03

6. SH3PX3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Gastroscyphus hectoris
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Alabes dorsalis
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Parvicrepis sp.
Genus C
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Cochleoceps spatula
Genus A
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Aspasmogaster tasmaniensis
Conidens laticephalus
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Dellichthys morelandi
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Pherallodus indicus
Discotrema crinophila
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Aspasmichthys alorensis
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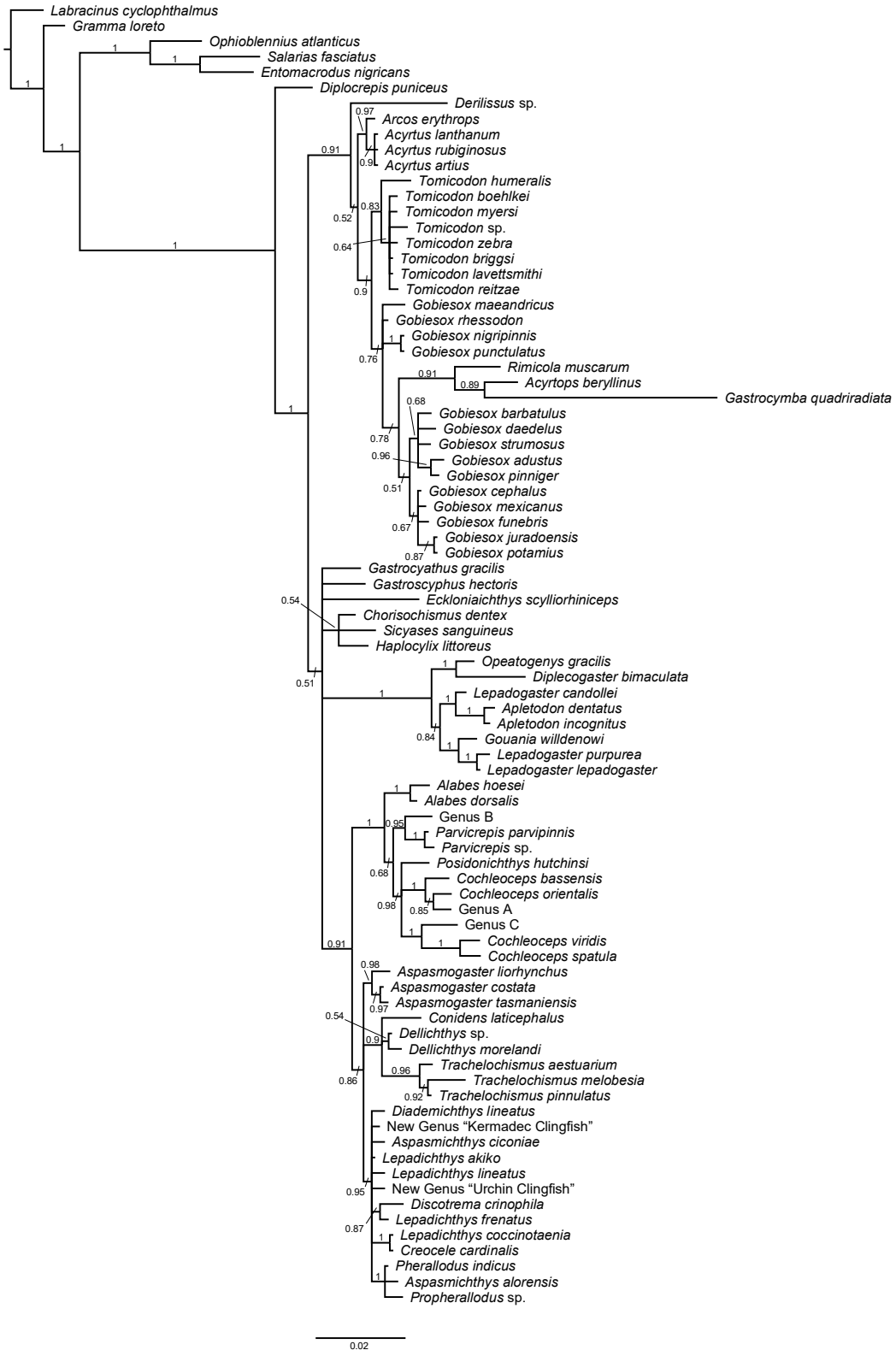
7. ZIC1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Acyrtus rubiginosus
Acyrtus artius
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Tomicodon boehlkei
Tomicodon myersi
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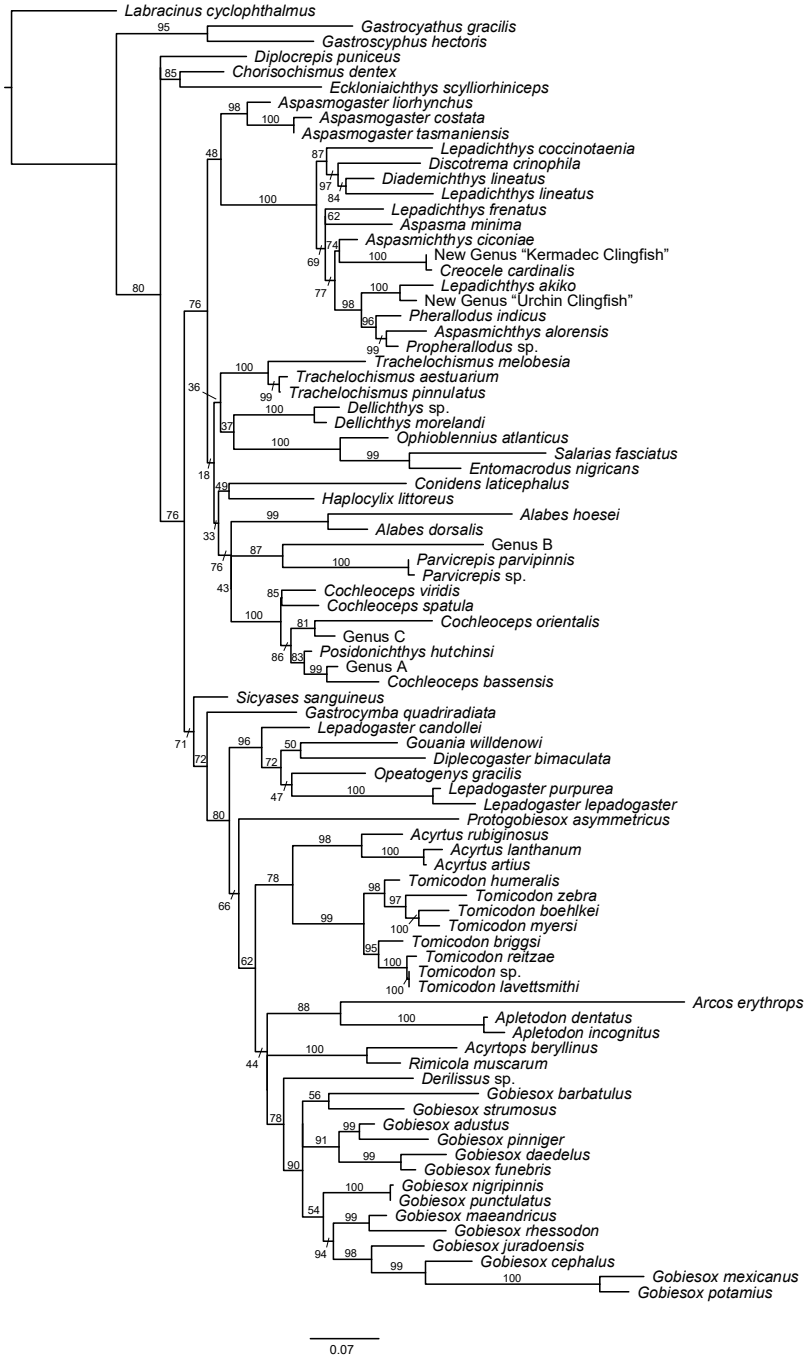
Tomicodon lavettsmithi
Tomicodon reitzae
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox nigripinnis
Gobiesox punctulatus
Rimicola muscarum
Acyrtops beryllinus
Gastrocymba quadriradiata
Gobiesox barbatulus
Gobiesox daedelus
Gobiesox strumosus
Gobiesox adustus
Gobiesox pinniger
Gobiesox cephalus
Gobiesox mexicanus
Gobiesox funebris
Gobiesox juradoensis
Gobiesox potamius
Gastrocyathus gracilis
Gastroscyphus hectoris
Eckloniaichthys scylliorhiniceps
Chorisochismus dentex
Sicyases sanguineus
Haplocylix littoreus
Opeatogenys gracilis
Diplecogaster bimaculata
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Gouania willdenowi
Lepadogaster purpurea
Lepadogaster lepadogaster
Alabes hoesei
Alabes dorsalis
Genus B
Parvicrepis parvipinnis
Parvicrepis sp.
Posidonichthys hutchinsi
Cochleoceps bassensis
Cochleoceps orientalis
Genus A
Genus C
Cochleoceps viridis
Cochleoceps spatula
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Conidens laticephalus
Dellichthys sp.
Dellichthys morelandi
Trachelochismus aestuarium
Trachelochismus melobesia
Trachelochismus pinnulatus
Diademichthys lineatus
New Genus “Kermadec Clingfish”
Aspasmichthys ciconiae
Lepadichthys akiko
Lepadichthys lineatus
New Genus “Urchin Clingfish”
Discotrema crinophila
Lepadichthys frenatus
Lepadichthys coccinotaenia
Creocele cardinalis
Pherallodus indicus
Aspasmichthys alorensis
Propherallodus sp.
0.02

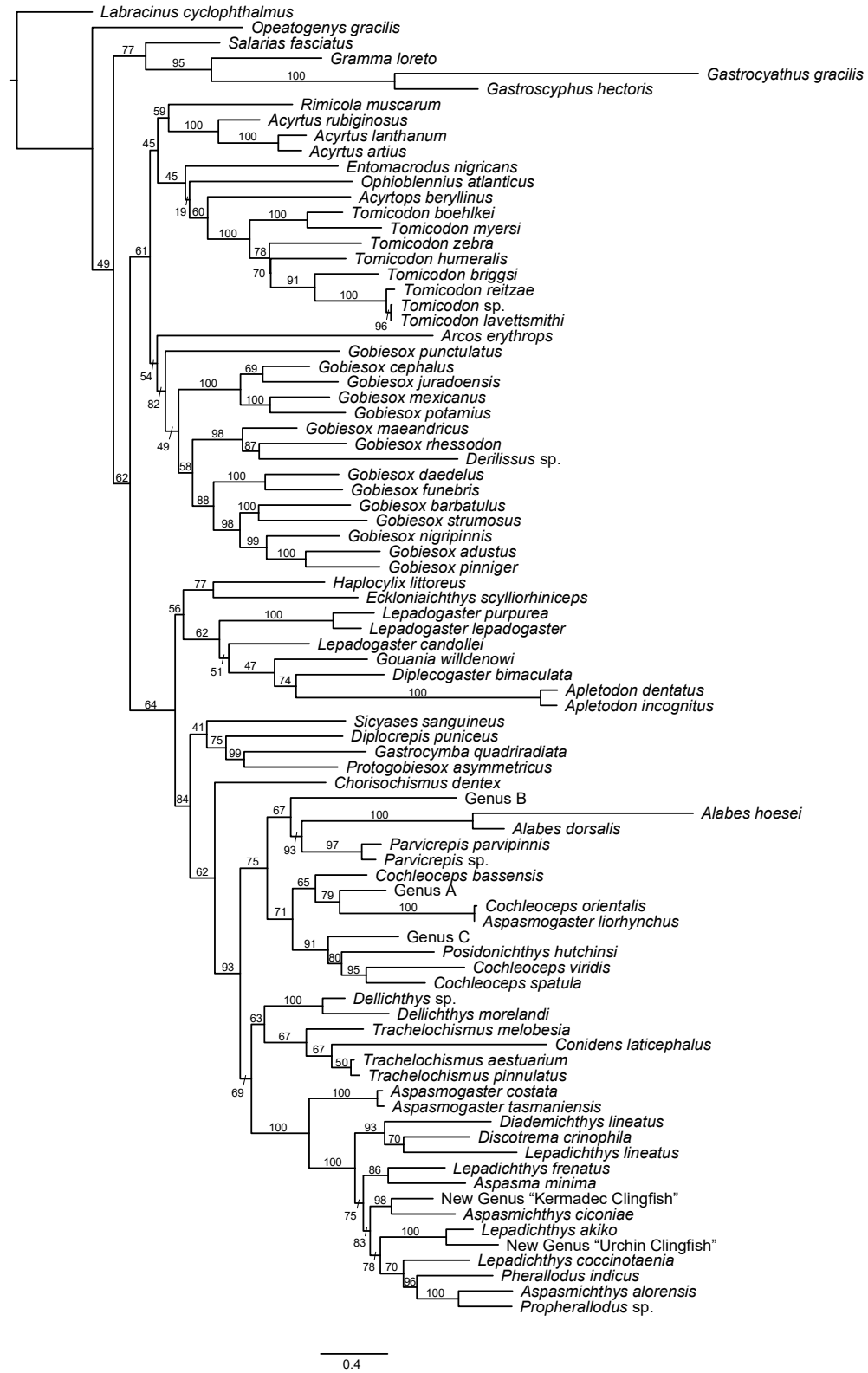
APPENDIX E

Phylograms obtained from the Maximum Likelihood analyses of the 12S (1), COI (2), ENC1 (3), GLYT (4), MYH6 (5), SH3PX3 (6), and ZIC1 (7) datasets. Numbers above branches represent bootstrap support values.

1. 12S



2. COI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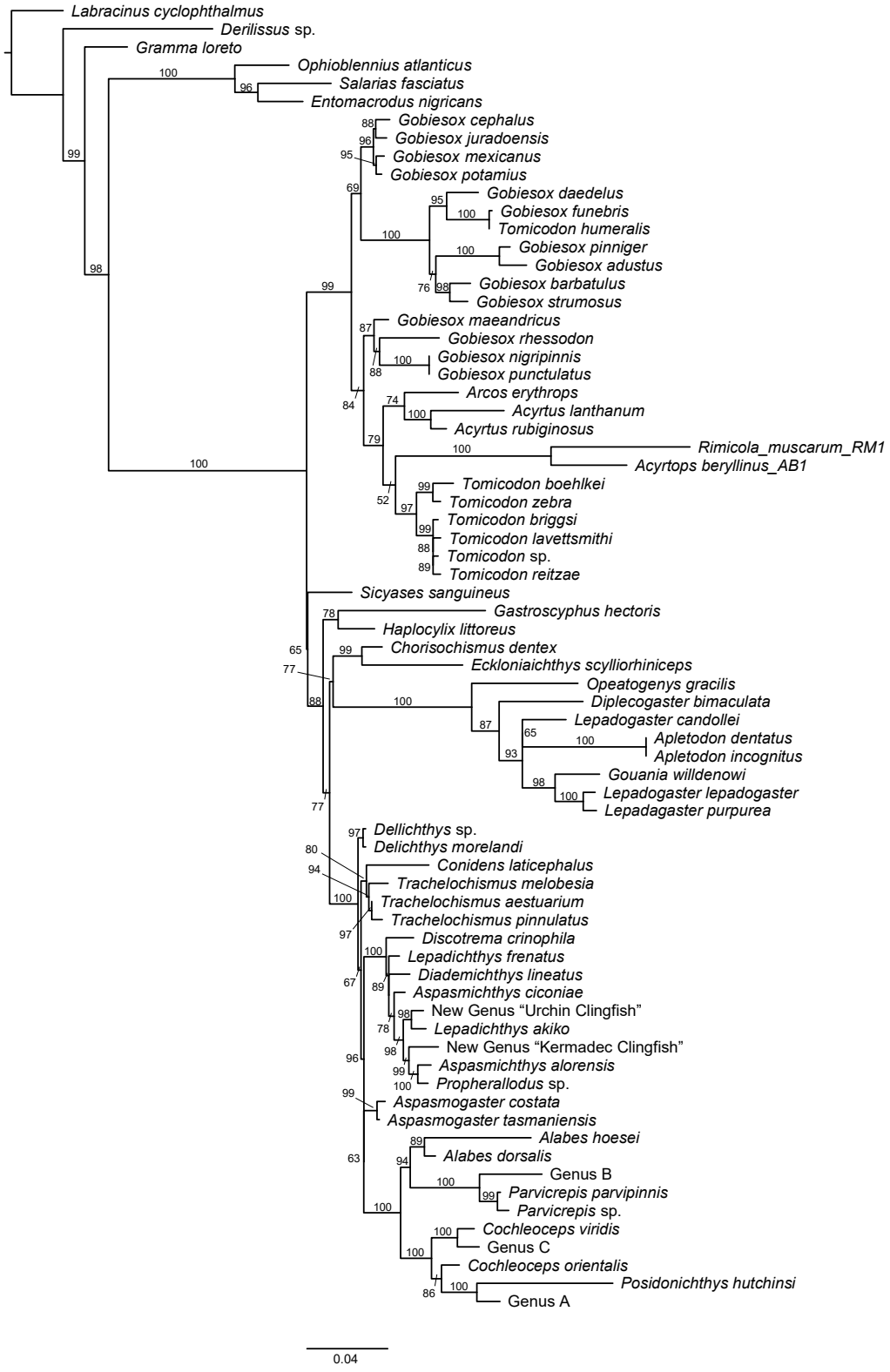
Opeatogenys gracilis
Salarias fasciatus
Gramma loreto
Gastrocyathus gracilis
Gastroscyphus hectoris
Rimicola muscarum
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Entomacrodus nigricans
Ophioblennius atlanticus
Acyrtops beryllinus
Tomicodon boehlkei
Tomicodon myersi
Tomicodon zebra
Tomicodon humeralis
Tomicodon briggsi
Tomicodon reitzae
Tomicodon sp.
Tomicodon lavettsmithi
Arcos erythrops
Gobiesox punctulatus
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox maeandricus
Gobiesox rhessodon
Derilissus sp.
Gobiesox daedelus
Gobiesox funebris
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox nigripinnis
Gobiesox adustus
Gobiesox pinniger
Haplocylix littoreus
Eckloniaichthys scylliorhiniceps
Lepadogaster purpurea
Lepadogaster lepadogaster
Lepadogaster candollei
Gouania willdenowi
Diplecogaster bimaculata
Apletodon dentatus
Apletodon incognitus
Sicyases sanguineus
Diplocrepis puniceus
Gastrocymba quadriradiata
Protogobiesox asymmetricus
Chorisochismus dentex
Genus B
Alabes hoesei
Alabes dorsalis
Parvicrepis parvipinnis
Parvicrepis sp.
Cochleoceps bassensis
Genus A
Cochleoceps orientalis
Aspasmogaster liorhynchus
Genus C
Posidonichthys hutchinsi
Cochleoceps viridis
Cochleoceps spatula
Dellichthys sp.
Dellichthys morelandi
Trachelochismus melobesia
Conidens laticephalus
Trachelochismus aestuarium
Trachelochismus pinnulatus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Diademichthys lineatus
Discotrema crinophila
Lepadichthys lineatus
Lepadichthys frenatus
Aspasma minima
New Genus “Kermadec Clingfish”
Aspasmichthys ciconiae
Lepadichthys akiko
New Genus “Urchin Clingfish”
Lepadichthys coccinotaenia
Pherallodus indicus
Aspasmichthys alorensis
Propherallodus sp.
0.4

3. ENC1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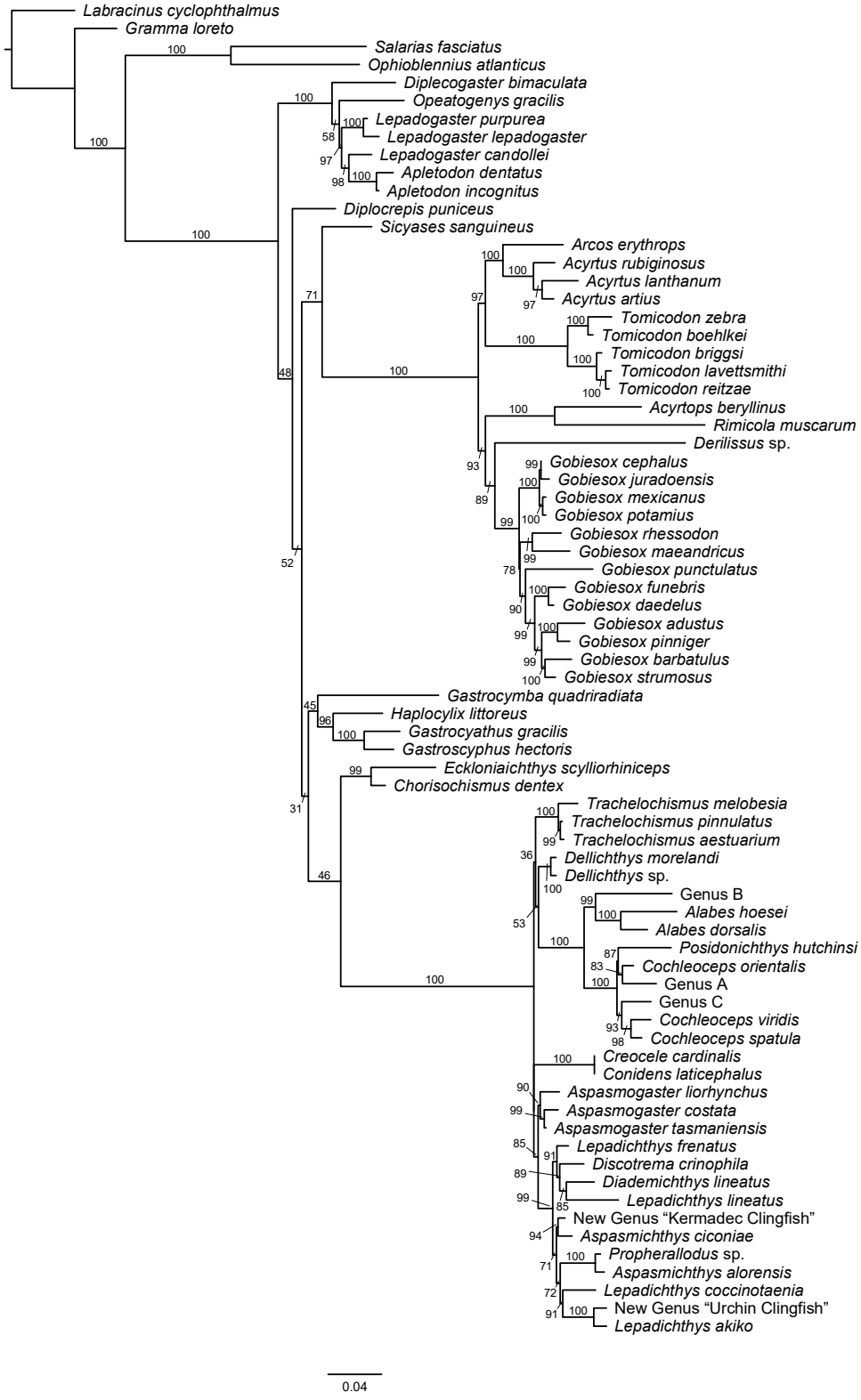
Salarias fasciatus
Entomacrodus nigricans
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox daedelus
Gobiesox funebris
Tomicodon humeralis
Gobiesox pinniger
Gobiesox adustus
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox nigripinnis
Gobiesox punctulatus
Arcos erythrops
Acyrtus lanthanum
Acyrtus rubiginosus
Rimicola_muscarum_RM1
Acyrtops beryllinus_AB1
Tomicodon boehlkei
Tomicodon zebra
Tomicodon briggsi
Tomicodon lavettsmithi
Tomicodon sp.
Tomicodon reitzae
Sicyases sanguineus
Gastroscyphus hectoris
Haplocylix littoreus
Chorisochismus dentex
Eckloniaichthys scylliorhiniceps
Opeatogenys gracilis
Diplecogaster bimaculata
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Gouania willdenowi
Lepadogaster lepadogaster
Lepadagaster purpurea
Dellichthys sp.
Delichthys morelandi
Conidens laticephalus
Trachelochismus melobesia
Trachelochismus aestuarium
Trachelochismus pinnulatus
Discotrema crinophila
Lepadichthys frenatus
Diademichthys lineatus
Aspasmichthys ciconiae
New Genus “Urchin Clingfish”
Lepadichthys akiko
New Genus “Kermadec Clingfish”
Aspasmichthys alorensis
Propherallodus sp.
Aspasmogaster costata
Aspasmogaster tasmaniensis
Alabes hoesei
Alabes dorsalis
Genus B
Parvicrepis parvipinnis
Parvicrepis sp.
Cochleoceps viridis
Genus C
Cochleoceps orientalis
Posidonichthys hutchinsi
Genus A
0.04

4. GLYT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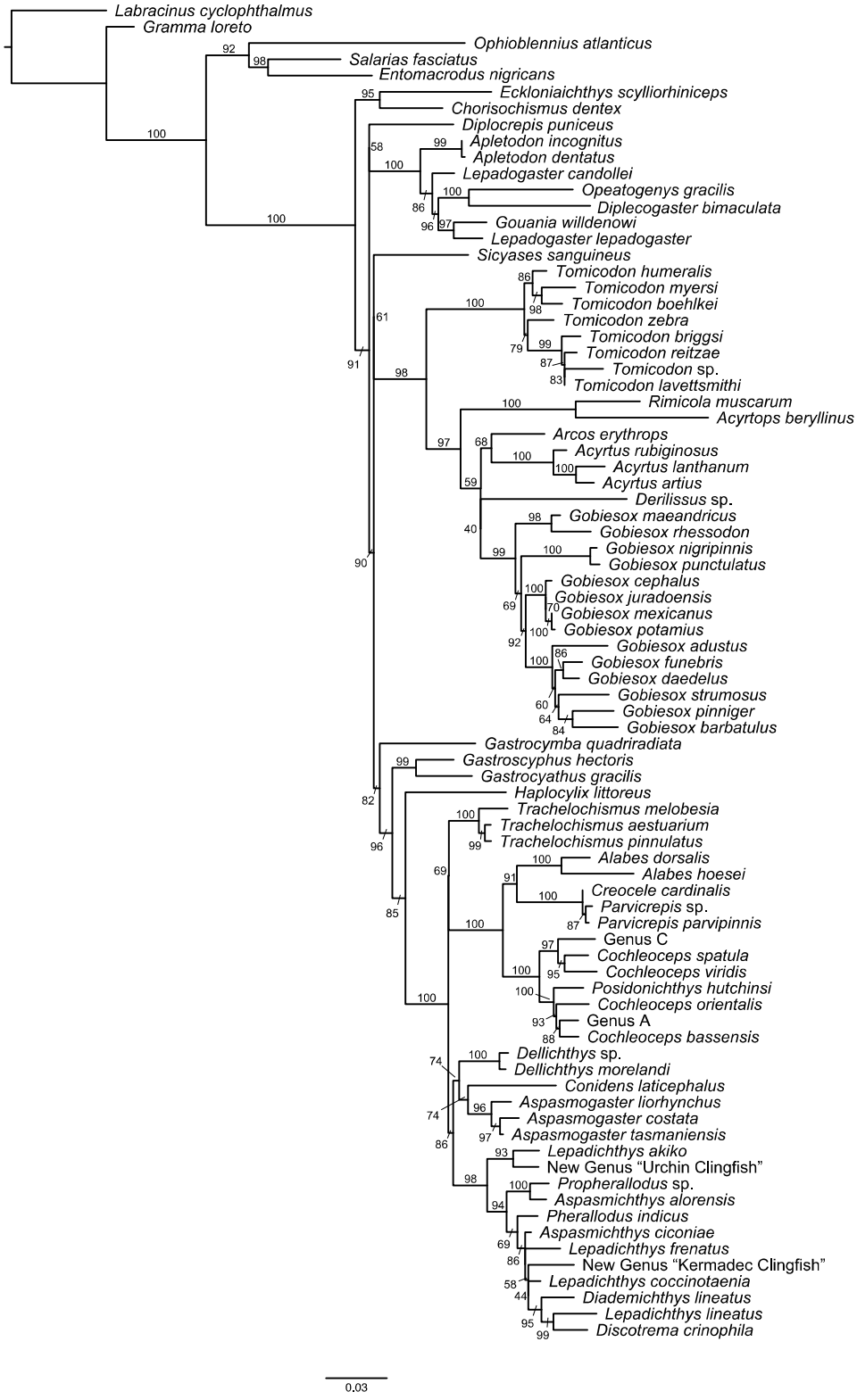
Ophioblennius atlanticus
Diplecogaster bimaculata
Opeatogenys gracilis
Lepadogaster purpurea
Lepadogaster lepadogaster
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Diplocrepis puniceus
Sicyases sanguineus
Arcos erythrops
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Tomicodon zebra
Tomicodon boehlkei
Tomicodon briggsi
Tomicodon lavettsmithi
Tomicodon reitzae
Acyrtops beryllinus
Rimicola muscarum
Derilissus sp.
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox rhessodon
Gobiesox maeandricus
Gobiesox punctulatus
Gobiesox funebris
Gobiesox daedelus
Gobiesox adustus
Gobiesox pinniger
Gobiesox barbatulus
Gobiesox strumosus
Gastrocymba quadriradiata
Haplocylix littoreus
Gastrocyathus gracilis
Gastroscyphus hectoris
Eckloniaichthys scylliorhiniceps
Chorisochismus dentex
Trachelochismus melobesia
Trachelochismus pinnulatus
Trachelochismus aestuarium
Dellichthys morelandi
Dellichthys sp.
Genus B
Alabes hoesei
Alabes dorsalis
Posidonichthys hutchinsi
Cochleoceps orientalis
Genus A
Genus C
Cochleoceps viridis
Cochleoceps spatula
Creocele cardinalis
Conidens laticephalus
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Lepadichthys frenatus
Discotrema crinophila
Diademichthys lineatus
Lepadichthys lineatus
New Genus “Kermadec Clingfish”
Aspasmichthys ciconiae
Propherallodus sp.
Aspasmichthys alorensis
Lepadichthys coccinotaenia
New Genus “Urchin Clingfish”
Lepadichthys akiko
0.04

5. MYH6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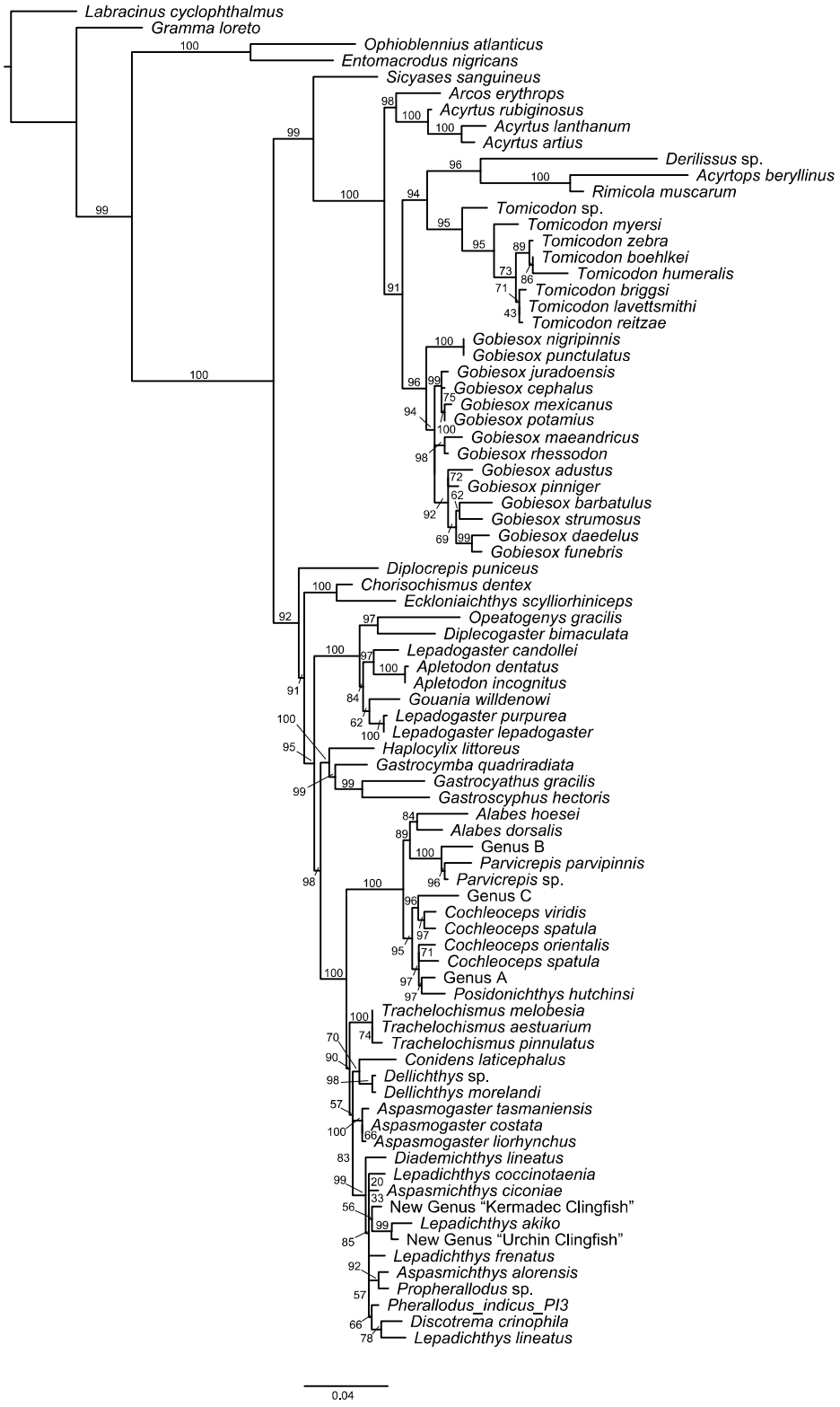
Ophioblennius atlanticus
Salarias fasciatus
Entomacrodus nigricans
Eckloniaichthys scylliorhiniceps
Chorisochismus dentex
Diplocrepis puniceus
Apletodon incognitus
Apletodon dentatus
Lepadogaster candollei
Opeatogenys gracilis
Diplecogaster bimaculata
Gouania willdenowi
Lepadogaster lepadogaster
Sicyases sanguineus
Tomicodon humeralis
Tomicodon myersi
Tomicodon boehlkei
Tomicodon zebra
Tomicodon briggsi
Tomicodon reitzae
Tomicodon sp.
Tomicodon lavettsmithi
Rimicola muscarum
Acyrtops beryllinus
Arcos erythrops
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Derilissus sp.
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox nigripinnis
Gobiesox punctulatus
Gobiesox cephalus
Gobiesox juradoensis
Gobiesox mexicanus
Gobiesox potamius
Gobiesox adustus
Gobiesox funebris
Gobiesox daedelus
Gobiesox strumosus
Gobiesox pinniger
Gobiesox barbatulus
Gastrocymba quadriradiata
Gastroscyphus hectoris
Gastrocyathus gracilis
Haplocylix littoreus
Trachelochismus melobesia
Trachelochismus aestuarium
Trachelochismus pinnulatus
Alabes dorsalis
Alabes hoesei
Creocele cardinalis
Parvicrepis sp.
Parvicrepis parvipinnis
Genus C
Cochleoceps spatula
Cochleoceps viridis
Posidonichthys hutchinsi
Cochleoceps orientalis
Genus A
Cochleoceps bassensis
Dellichthys sp.
Dellichthys morelandi
Conidens laticephalus
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Lepadichthys akiko
New Genus “Urchin Clingfish”
Propherallodus sp.
Aspasmichthys alorensis
Pherallodus indicus
Aspasmichthys ciconiae
Lepadichthys frenatus
New Genus “Kermadec Clingfish”
Lepadichthys coccinotaenia
Diademichthys lineatus
Lepadichthys lineatus
Discotrema crinophila
0.03

6. SH3PX3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Ophioblennius atlanticus
Entomacrodus nigricans
Sicyases sanguineus
Arcos erythrops
Acyrtus rubiginosus
Acyrtus lanthanum
Acyrtus artius
Derilissus sp.
Acyrtops beryllinus
Rimicola muscarum
Tomicodon sp.
Tomicodon myersi
Tomicodon zebra
Tomicodon boehlkei
Tomicodon humeralis
Tomicodon briggsi
Tomicodon lavettsmithi
Tomicodon reitzae
Gobiesox nigripinnis
Gobiesox punctulatus
Gobiesox juradoensis
Gobiesox cephalus
Gobiesox mexicanus
Gobiesox potamius
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox adustus
Gobiesox pinniger
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox daedelus
Gobiesox funebris
Diplocrepis puniceus
Chorisochismus dentex
Eckloniaichthys scylliorhiniceps
Opeatogenys gracilis
Diplecogaster bimaculata
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Gouania willdenowi
Lepadogaster purpurea
Lepadogaster lepadogaster
Haplocylix littoreus
Gastrocymba quadriradiata
Gastrocyathus gracilis
Gastroscyphus hectoris
Alabes hoesei
Alabes dorsalis
Genus B
Parvicrepis parvipinnis
Parvicrepis sp.
Genus C
Cochleoceps viridis
Cochleoceps spatula
Cochleoceps orientalis
Cochleoceps spatula
Genus A
Posidonichthys hutchinsi
Trachelochismus melobesia
Trachelochismus aestuarium
Trachelochismus pinnulatus
Conidens laticephalus
Dellichthys sp.
Dellichthys morelandi
Aspasmogaster tasmaniensis
Aspasmogaster costata
Aspasmogaster liorhynchus
Diademichthys lineatus
Lepadichthys coccinotaenia
Aspasmichthys ciconiae
New Genus "Kermadec Clingfish"
Lepadichthys akiko
New Genus "Urchin Clingfish"
Lepadichthys frenatus
Aspasmichthys alorensis
Propherallodus sp.
Pherallodus_indicus_PI3
Discotrema crinophila
Lepadichthys lineatus
0.04

7. ZIC1


Labracinus cyclophthalmus
Gramma loreto
Ophioblennius atlanticus
Salarias fasciatus
Entomacrodus nigricans
Diplocrepis puniceus
Opeatogenys gracilis
Diplecogaster bimaculata
Lepadogaster candollei
Apletodon dentatus
Apletodon incognitus
Gouania willdenowi
Lepadogaster purpurea
Lepadogaster lepadogaster
Derilissus sp.
Arcos erythrops
Acyrtus lanthanum
Acyrtus rubiginosus
Acyrtus artius
Acyrtops beryllinus
Rimicola muscarum
Tomicodon humeralis
Tomicodon myersi
Tomicodon boehlkei
Tomicodon sp.
Tomicodon briggsi
Tomicodon lavettsmithi
Tomicodon zebra
Tomicodon reitzae
Gobiesox maeandricus
Gobiesox rhessodon
Gobiesox nigripinnis
Gobiesox punctulatus
Gobiesox barbatulus
Gobiesox strumosus
Gobiesox daedelus
Gobiesox adustus
Gobiesox pinniger
Gobiesox cephalus
Gobiesox mexicanus
Gobiesox funebris
Gobiesox juradoensis
Gobiesox potamius
Sicyases sanguineus
Chorisochismus dentex
Eckloniaichthys scylliorhiniceps
Haplocylix littoreus
Gastrocyathus gracilis
Gastroscyphus hectoris
Alabes hoesei
Alabes dorsalis
Genus B
Parvicrepis parvipinnis
Parvicrepis sp.
Genus C
Cochleoceps viridis
Cochleoceps spatula
Posidonichthys hutchinsi
Cochleoceps bassensis
Cochleoceps orientalis
Genus A
Conidens laticephalus
Dellichthys sp.
Dellichthys morelandi
Gastrocymba quadriradiata
Trachelochismus aestuarium
Trachelochismus melobesia
Trachelochismus pinnulatus
Aspasmogaster liorhynchus
Aspasmogaster costata
Aspasmogaster tasmaniensis
Lepadichthys lineatus
Discotrema crinophila
Lepadichthys frenatus
Propherallodus sp.
Pherallodus indicus
Aspasmichthys alorensis
Lepadichthys akiko
Diademichthys lineatus
Aspasmichthys ciconiae
New Genus “Kermadec Clingfish”
New Genus “Urchin Clingfish”
Lepadichthys coccinotaenia
Creocele cardinalis
0.03

APPENDIX F

Species tree estimated using StarBEAST. Numbers above branches represent the posterior probabilities.